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DIFFERENTIAL INJURY WITHIN VARIETIES, INBRED LINES, AND HYBRIDS OF FIELD CORN CAUSED BY THE CORN EARWORM, HELIOTHIS ARMIGERA (HBN.)[1]

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INTRODUCTION

In the United States the corn earworm (*Heliothis armigera* (Hbn.)) is one of the most destructive of insect pests. Each year it causes severe losses, especially in the Central and Southern States, in tomatoes, soybeans, and cotton, as well as in corn. In field corn the earworm is almost impossible to control at the present time. The small unit value of the crop and the large acreage make the use of insecticides impractical; hence the principal methods of control must consist in cultural practices or biological control. The most promising possibility of success appears to lie in the discovery and use of resistant strains.

The present study was undertaken to determine the range of injury caused by the earworm to various strains and varieties of corn and to classify the varieties, hybrids, and inbreds on the basis of the amount of injury sustained. It was expected that this information would eventually be used in a breeding program directed toward a reduction of the losses from this insect in field corn (*Zea mays* L.)

REVIEW OF LITERATURE

There has been considerable study of the resistance of field and sweet corn to the corn earworm and of the factors associated with this resistance. Much of this study has been devoted to sweet corn because of the greater severity of the injury in sweet corn and the higher unit value of the crop. Until recently, when the problem was partly solved by the use of resistant varieties, this insect was the major factor (*4*)[3] in preventing the profitable growing of sweet corn in the southern part of the United States.

The first detailed study of resistance to corn earworm and one of the first attempts to use plant hybridization in insect control was made by Collins and Kempton (*4*). Their paper presents the most extensive and thorough study so far reported. Some of the facts or probable relationships brought out in this paper are: (1) Resistance to corn earworm may be transmitted from field corn to sweet corn. (2) Low damage was significantly correlated with a number of mor-

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[2] The authors are indebted to R. W. Jugenheimer for permission to use some data collected in 1938, and to various members of the Departments of Agronomy and Entomology for helpful suggestions and criticisms. The following as research assistants were among those who aided in the collection of detailed data: Merle W. Allen, R. O. Snelling, R. G. Dahms, A. L. Robinson, Everett Blood, and Stewart Schell.

[3] Italic numbers in parentheses refer to Literature Cited, p. 99.

phological characters which are mostly interrelated. The greatest correlation was with prolongation of husk, followed by many layers of husk and few husk leaves. (3) Resistant progenies were low both in number of larvae and damage per larva, possibly indicating that plants avoided by moths were also less acceptable to larvae and "that at least a part of the immunity is the result of chemical differences, perhaps the presence of some volatile substance distasteful alike to the moth and the larva." (4) "No difficulty was experienced in securing by hybridization and selection the desired plant characters in combination with the seed characters of sweet corn."

A number of workers have studied, and some have emphasized, the importance of long husks as a protection against corn earworm damage (*2, 3, 5, 6, 8, 9, 11, 15, 16, 18, 19*). Some of these workers also mention the importance of tightness of husk. Phillips and Barber (*16*) studied these characters in considerable detail in 13 varieties. Considering all years and all varieties, the ears with short loose husks had an average of 39.14 kernals injured per ear and those with long tight husks had an average of 18.79, a difference of 20.35 kernels per ear in favor of the latter class. The averages of the other classes were strictly intermediate. Phillips and Barber do not discuss the still greater intervarietal differences in damage shown by their data, which appear to indicate that differences between varieties may be of more importance than difference in husk type (*16, table 3*).

McColloch (*12, 13, 14*) showed that differences in infestation of four varieties could be partly accounted for on the basis of relation of time of silking or stage of maturity to number of eggs. He found that fewer eggs were deposited on plants lacking rough and hairy leaves, and he mentioned the attractiveness of the silks as a character bearing on resistance. This factor has also been studied by Phillips and Barber (*17*). Barber (*1*) studied the relationship between the strong cannibalistic tendencies of corn earworm larvae and the effect of long tight husks in confining the larvae of various instars within a small space where the larger apparently seek out, kill, and feed on the smaller ones. Isely (*10*) has shown that different plants and even parts of the same plant are not of equal value as larval food when measured by size, length of life, or adult fecundity. These and other shorter papers record the following as the chief mechanisms affecting the resistance of corn to corn earworm:

Length of husk, cannibalism of larvae.
Tightness of husks.
Number of husk leaves.
Number of husk layers.
Male florets at tip of ear.
Synchronization of time of silking and peak of oviposition.
Hairiness of plant.
Length of silking period; number of ears per plant.
Total leaf area available for oviposition.
Attractiveness of plant for oviposition.
Value as food for larvae.

Dungan and his associates (*7*) have mentioned certain field corn inbreds that transmit resistance to earworm in Illinois.

MATERIAL AND METHODS

There is reported here information gained at the Kansas station concerning resistance of corn to the corn earworm since 1924. The

earlier entomological investigations were conducted by the late Prof. J. W. McColloch; those since 1928 have been under the direction of the senior author. In Kansas the corn earworm usually has three generations and a partial fourth, and though frequently these are not distinct, the damage and infestation in corn and other host plants increases more or less uniformly in severity toward the latter part of the season. In most years only an occasional ear of corn escapes infestation. The number of ears studied during various years, the percentage of infestation, and other pertinent data are recorded in table 1.

TABLE 1.—*Intensity of corn earworm infestation, Manhattan, Kans., 1929–37*

[All varieties and hybrids]

Year	Ears		Infested	Average class of injury
	Total	Uninfested		
	Number	*Number*	*Percent*	
1929	7,058	1,506	78.7	(1)
1931	20,159	46	99.9	3.5
1932	6,247	623	90.0	2.2
1933	19,697	317	98.4	2.4
1934 [2]				
1935	5,937	66	98.9	3.5
1936 [3]				
1937	14,484	80	99.4	3.4
Total	73,582	2,638	96.4	3.0

[1] Not used.
[2] Corn killed by drought before ears matured.
[3] Corn too badly damaged by grasshoppers and drought for records

The plant material used has been chiefly that grown in the breeding and yield-testing experiments of the Department of Agronomy, supplemented by a few special experiments. Examination for earworm damage has usually been made at the time these plots were harvested. In the earlier years only the percentage of ears infested was recorded. Later it became apparent that even where all ears of two strains were infested they might differ markedly in the average amount of damage done. Since 1930 the amount of damage on each ear has been determined and the ears from each strain classified into six groups. Class 1 contained those ears with no evident infestation; class 6, those with the greatest damage. In addition to the direct damage to kernels some weight was also given to poorly filled ears in cases where the condition of such ears appeared to be due to the cutting of silk by the corn earworm with consequent prevention of fertilization. Where feeding was done on the hardened kernels the damage was graded severely. The classification was kept approximately the same from year to year by the use of a photograph showing characteristic ears in each class (fig. 1). Later the data gathered in the field were used in calculating the average "class of injury" for each plot.

It is evident that a more detailed record, such as the number of kernels damaged per ear, would have been desirable, but with the limited funds and time available this was not practical. A large plot devoted entirely to corn earworm study where the infestation and damage could have been studied as each strain reached maturity also

would have been desirable. It is known that several possible sources of error were present, particularly the possibility of mistaking the damage of other insects for that of the corn earworm and the difficulty of classifying the nubbins which predominated in various strains during some of the drought years. The necessity for waiting for harvesttime also introduced some sources of error. On the other hand, the refined estimate of damage used, the relatively large samples available in some cases, and the opportunity of obtaining information on the actual strains being used in breeding work, as well as the possibility of studying large numbers of strains, are believed to have outweighed the disadvantages mentioned above. Since the study was largely exploratory the method is believed to have been satisfactory.

The hybrid and inbred material studied has thus not been selected consciously for resistance to earworm, but is rather a more or less

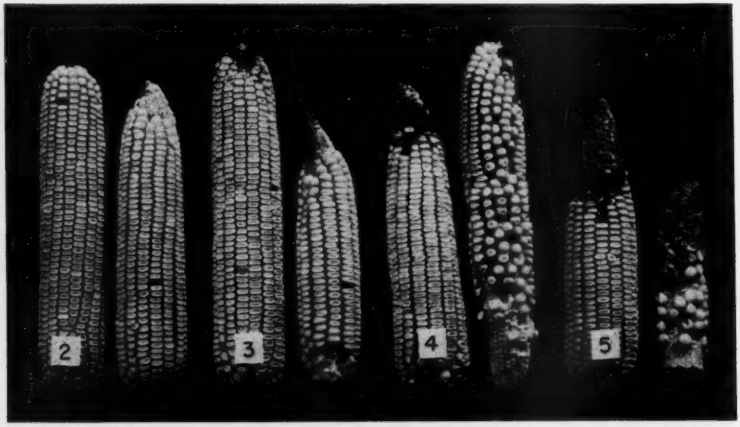

FIGURE 1.—The photograph used in classifying ears for corn earworm damage: Class 1 (not shown), no injury; classes 2 to 5, progressively greater injury; class 6 (not shown), all ears more severely injured.

random sample of the corn germ plasm being used for corn improvement at the Kansas station. A carefully controlled selection for extremes of earworm damage undoubtedly would have produced wider differences even in the absence of detailed information regarding the mechanisms of resistance.

The range of variation found among the different strains studied has sometimes been small, but in those tests in which statistical analysis was possible significant differences have been found to exist. From year to year there has been considerable difference in the average class of injury. Where it has been possible to get information there appears to be a parallel between this measure of damage and the percentage of ears infested.

In 3 years, 1929, 1934, and 1935, a severe early infestation occurred in the agronomy nursery. The feeding of earworms on the opening curl resulted in "ragworm" or "budworm" damage. The percentage of plants damaged was recorded for each strain.

The number of replications studied varied considerably from year to year and in different experiments. Two types of replications are available for study. In many tests the exact strain was repeated from 2 to 10 times and data were secured on each replication. In tests involving single crosses it has been possible to average all strains containing the same parent, thus obtaining replication and a measure of the average performance of an inbred in its hybrids. A similar procedure was followed with double and three-way crosses. Each plot studied usually consisted of 2 rows of 10 hills each so that approximately 40 ears were harvested and rated in regard to class of injury. In the study of the performance of individual inbreds in single crosses the final average was frequently based on several hundred ears. Unless otherwise stated, the term "hybrid" as used in this paper refers to a first generation cross between two inbred lines.

EXPERIMENTAL RESULTS

OPEN-POLLINATED VARIETIES

During the earlier years open-pollinated varieties were the principal objects of study. The infestation and damage to ears of nine of the varieties occurring most frequently are recorded in table 2.

TABLE 2.—*Infestation and damage to ears of open-pollinated varieties at Manhattan, Kans., in certain years from 1925 to 1933* [1]

Variety	Ears infested			1931		1932		1933		Average ear infestation	Pride of Saline ear infestation in same tests	Average class of injury 1931 to 1933
	1925	1927	1929	Ears infested	Average class of injury	Ears infested	Average class of injury	Ears infested	Average class of injury			
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Per cent*		*Per cent*		*Per cent*	*Per cent*	
Hays Golden	------	64.3	68.3	100	2.98	88.3	2.03	95.4	2.16	83.3	88.5	2.39
Reid Yellow Dent	58.4	55.4	76.1	100	3.10	85.6	1.91	97.1	2.17	78.8	86.8	2.39
Harmon White	------	------	84.9	100	3.29	90.7	2.11	100.0	2.22	93.9	95.1	2.54
Freed White	69.8	62.0	72.0	100	3.23	88.1	2.14	93.4	2.28	80.9	86.8	2.55
Cassel White	66.2	72.0	66.1	100	3.00	88.7	2.12	100.0	2.66	82.3	86.8	2.59
Colby Bloody Butcher	64.5	69.9	57.6	100	3.29	90.4	2.09	100.0	2.44	80.4	86.8	2.61
Pride of Saline	77.1	63.2	84.5	100	3.41	95.9	2.22	100.0	2.33	86.8	------	2.65
Midland Yellow Dent	77.3	44.1	86.1	100	3.68	88.1	2.15	96.4	2.27	82.0	86.8	2.70
Boone County White	------	57.2	76.8	100	4.62	94.4	2.29	------	------	82.1	85.7	[2] 3.46

[1] Arranged in order of average class of injury.
[2] 2-year average.

Studied were made of these varieties in 1925, 1927, 1929, 1931, 1932, and 1933. In order to provide a convenient means of evaluating the data, the average infestation of each variety has been compared with the average of Pride of Saline in the same tests. This method must be used with some caution since the relative infestation in some cases varied with the year and severity of infestation. Strictly speaking, only those tests conducted in the same season are comparable. In interpreting the data, no serious errors will be made if this fact is kept in mind.

It is evident that the infestation on all varieties was much heavier during the last 3 years than during the first 3. The average percentage of ears infested for the 6 years indicates that Pride of Saline had the highest infestation of any variety represented in all 6 years, but the difference of 8 percent between this variety and Reid Yellow Dent,

the variety with the lowest infestation, is probably of no great importance.

The most obvious relationship between infestation or damage is that with stage of maturity, particularly with the date at which flowering or silking takes place. During the earlier years this maturity relationship was measured in terms of the days from planting until the time when one-tenth or one-eighth of the plants in a given plot were shedding pollen. Pollen shedding has been shown to be very highly correlated with the appearance of silks, and hence with the availability of silks, which are the preferred place of oviposition for the corn earworm moths. In the study of scatter diagrams of the data gathered during the several years there appeared to be a direct relationship between time of flowering as measured by pollen shedding and both percentage of infestation and class of injury. In 1929, when the largest number of varieties were available for study, the correlation between date of one-tenth pollen shedding and percentage of ears infested was $r=0.54$ for the 45 entries. This correlation is considered highly significant.[4] The data for the years 1931 and 1932, in which 37 and 26 entries, respectively, were studied, show a similar relationship between the time of pollen shedding and the average class of injury. In the other years somewhat fewer varieties were available for study.

From these data, obtained with open-pollinated varieties and gathered under the severe conditions of infestation in Kansas, there appears to be no very dependable difference of practical importance that can be measured independently of the date of pollen shedding or silking. This does not necessarily mean that there are no real differences bebetween the different varieties. It seems reasonable to assume that the great variability within each variety obscures any real differences that might be demonstrated under lower infestation and with larger samples, since susceptible strains within the varieties would render them especially sensitive to the synchronization of time of silking with the peak of moth oviposition. The belief that inbred strains and their hybrids would be more favorable material for study, and the gradual increase of interest in corn hybrids, led to the abandonment of further study of varieties. In recent years emphasis has been placed on the various commercial and experimental hybrids.

INBREDS AND HYBRIDS WITH REFERENCE TO EAR INJURY

Relation of Class of Ear Injury to Stage of Flowering

Since 1930 many different hybrids in the agronomy testing nursery have been studied, but only a part of the data are here reported. These hybrids consist of single crosses, three-way and four-way crosses, and top crosses. Most of the data presented below concern single crosses since these tend to be more uniform and to give a better measure of the performance of the respective inbreds. The ability of an inbred to increase the resistance in the hybrids in which it occurs is the primary object of interest in these studies. Much of the information gained from hybrids other than single crosses has been used along with other data in selecting the more desirable combinations for the practical corn-breeding project.

Since the hybrids studied have depended on the time and material available, the number of replications and the number of hybrids

[4] In testing the significance of the correlation coefficients, use has been made of table 7.2 by Snedecor (*20*).

involved have varied greatly from year to year. Practically all of the hybrids studied have come from the breeding and testing nursery and from a few small plots planted especially for the study of corn earworm infestation. In the agronomy nursery the hybrids were usually grouped on the basis of similarity of origin or time of maturity. In the various tests in which replications and arrangement of plots permitted the use of statistical methods, a minimum difference of from

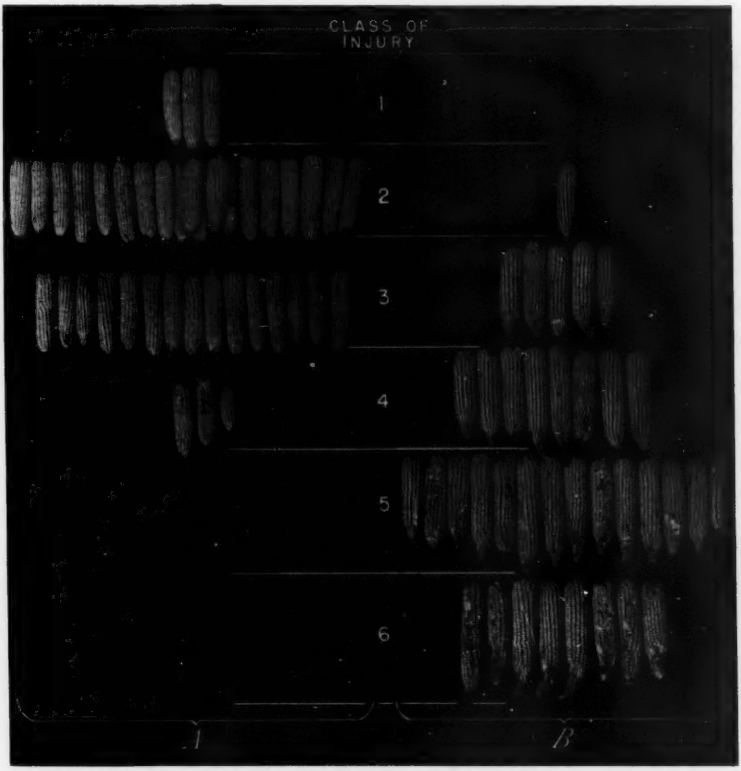

FIGURE 2.—All corn produced in 1938 by plots of two single-cross hybrids graded according to class of injury and showing contrasting earworm injury. The plots were located on opposite sides of a 3-foot alley: *A*, Row 16, Ks. Hy. 126 × Ks. Y. S. 151, average class of injury 2.45, three uninfested ears; *B*, row 103, Ks. M. 201 × Ind. 38–11, average class of injury 4.64, frequent damage far down on ear, 3 days later in flowering than row 16. Numerals on figure indicate class of injury.

0.5 to 1.0 class of injury has been found to be significant. The consistent difference between the hybrids with light injury and those with severe injury, as shown in figures 2 and 3, is also certainly of economic importance.

In all of the years the date of flowering, as represented by the appearance of silks or the shedding of pollen, has been available for com-

parison with the amount of earworm injury. Pollen shedding and silking are known to be highly correlated, and both characters have been used as a measure of the relation between time of silking and amount of damage, thereby giving some idea of the importance in various strains of the synchronization of time of silking and peak of moth oviposition. In 1931 the extension of the husk beyond the ear tip was determined for each of 20,159 ears in a study of the effect of this variable on earworm damage.

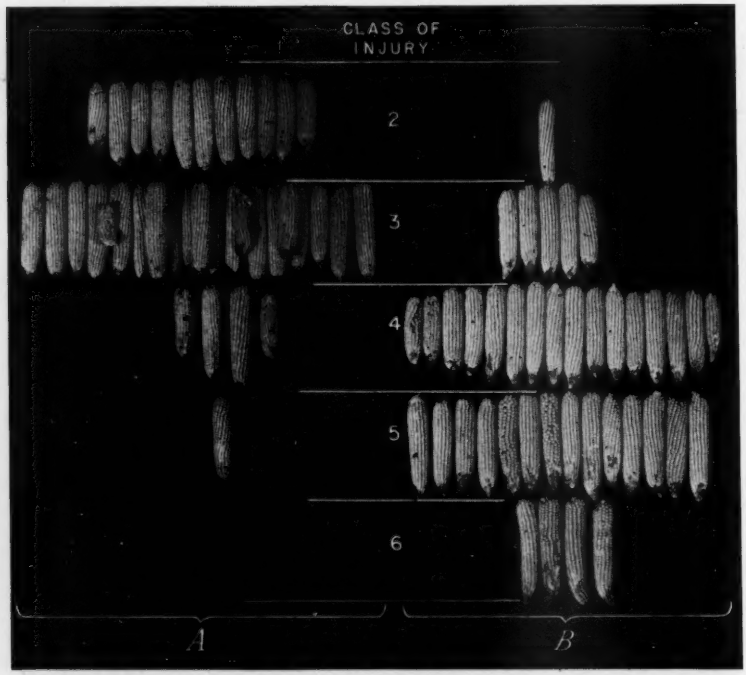

FIGURE 3.—Two single-cross hybrids graded according to class of injury and showing contrast in earworm injury. Both hybrids have all ears injured and have one parent in common: *A*, Row 123, Ks. Hy. 126 × Ks. Y. S. 175, average class of injury 2.86; *B*, row 121, Ks. Hy. 126 × Ks. Y. S. 167, average class of injury 4.38, severe injury to hardened kernels does not show well in the photograph; 1 day earlier in flowering than row 123. Numerals on figure indicate class of injury.

In a group of 100 early single crosses in 1931 there was a range of average class of injury from 2.70 to 4.61. Pollen shedding took place in this experiment between the dates July 4 and 17. A study of the correlation between the average class of injury and the days from planting until one-eighth of the plants in the respective plots were shedding pollen, appeared to show no relationship ($r=-0.05$). A study of the scatter diagram and of records of the hybrids with the highest and lowest average class of injury (table 3) suggests the slight possibility of a curvilinear relationship between these two characters.

TABLE 3.—*Husk extension, time of flowering, and class of earworm injury, in 12 slightly and 12 heavily injured hybrids from a group of 100 early single crosses and of 149 Pride of Saline single crosses studied at Manhattan, Kans., 1931*

100 EARLY SINGLE CROSSES

Row No.	Hybrid	Husk extension			Open husks	Ears showing indicated class of injury						Total ears	Average class of injury	Time from planting to ⅛ pollen shedding
		Average	High	Low		1	2	3	4	5	6			
		Centimeters	*Centimeters*	*Centimeters*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*		*Days*
59	17 × 16	+3.40	7	−1		0	7	16	0	0	0	23	2.70	62
105	36 × 22	+1.55	5	−5	1	0	8	26	1	0	0	35	2.80	63
39	12 × 22	+2.55	3	−3	1	0	15	14	8	0	0	37	2.81	62
25	9 × 11	−.85	5	−5	1	7	14	8	21	0	0	50	2.86	59
91	29 × 22	+4.65	6	2		0	12	21	7	0	0	40	2.88	61
43	13 × 26	+3.60	6	1		0	6	27	2	0	0	35	2.89	59
37	12 × 13	+3.70	7	0		0	9	24	3	1	0	37	2.89	58
98	34 × 35	+2.90	6	0		0	13	24	8	0	0	45	2.89	50
33	10 × 35	+2.10	6	−4		0	12	30	7	0	0	49	2.90	60
45	13 × 14	+4.05	6	2		0	6	33	3	0	0	42	2.93	58
72	22 × 37	+.80	5	−4		0	16	23	7	0	2	48	2.94	62
69	20 × 27	−1.40	6	−6		0	5	18	2	1	0	26	2.96	61
	Average	+2.25	5.8	−2									2.87	59.6
	Freed check [1]		14	−8									3.60	
87	27 × 26	+4.20	8	0		0	0	4	17	10	0	31	4.19	60
106	37 × 23	−.25	5	−4		0	1	18	6	2	13	40	4.20	64
93	32 × 35	+3.10	5	1		0	0	4	24	15	0	43	4.25	62
7	5 × 30	−3.10	7	−7	5	0	0	5	25	13	2	45	4.27	55
1	1 × 13	+1.15	5	−5	2	0	2	7	15	8	7	39	4.28	55
75	23 × 22	−.30	6	−5		0	3	9	10	3	12	37	4.32	64
3	5 × 1	−1.95	9	−7	11	1	2	8	13	20	6	50	4.37	53
5	5 × 14	+.50	3	−2		0	0	4	22	12	4	42	4.38	57
94	33 × 37	+2.95	6	−5		0	0	6	21	7	0	34	4.39	62
51	15 × 23	−2.40	3	−5		0	0	6	17	15	4	42	4.40	62
74	23 × 18	−2.25	6	−6		0	1	9	12	4	13	39	4.49	64
73	23 × 8628	−4.50	−1	−7		0	0	8	16	15	12	51	4.61	65
	Average	−.24	5.2	−4.3									4.35	60.3

[1] 12 rows.

TABLE 3.—*Husk extension, time of flowering, and class of earworm injury, in 12 slightly and 12 heavily injured hybrids from a group of 100 early single crosses and of 149 Pride of Saline single crosses studied at Manhattan, Kans., 1931*—Continued

100 EARLY SINGLE CROSSES—Continued

Row No.	Hybrid	Husk extension			Open husks	Ears showing indicated class of injury						Total ears	Average class of injury	Time from planting to ⅛ pollen shedding
		Average	High	Low		1	2	3	4	5	6			
		Centimeters	*Centimeters*	*Centimeters*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*		*Days*
8	5 × 14	+6.65	11	4		0	21	12	0	0	0	33	2.36	68
98	41 × 53	+4.40	13	−2		0	15	15	0	0	0	30	2.50	64
28	10 × 17	+2.30	4	−3		0	9	25	0	0	0	34	2.74	64
11	5 × 19	+2.10	5	−1		0	9	26	0	0	0	35	2.74	67
78	27 × 34	+10.45	13	6		0	8	24	0	0	0	32	2.75	69
23	9 × 12	+5.45	9	3		0	4	24	0	0	0	28	2.86	67
2	2 × 11	+3.40	7	1		0	12	23	7	0	0	42	2.88	65
144	48 × 56	+5.60	9	3		0	9	10	6	0	0	25	2.88	68
18	8 × 14	+3.85	8	−1		0	8	28	4	0	0	40	2.90	66
9	5 × 17	+5.10	8	4		0	5	26	2	0	0	33	2.91	65
15	7 × 18	+9.10	13	6		0	3	36	0	0	0	39	2.92	66
141	48 × 52	+5.95	11	3		0	6	15	4	0	0	25	2.92	66
	Average	5.36	9.25	1.9									2.78	66.3
	Pride of Saline checks [2]												3.70	
135	47 × 57	+4.60	9	0		0	0	12	12	1	10	35	4.26	65
124	45 × 52	+8.35	13	7		0	0	5	16	16	0	37	4.30	67
75	26 × 39	−1.75	1	−4	12	0	0	2	24	15	0	41	4.32	69
156	50 × 55	+4.65	7	−3	2	0	0	9	8	5	8	30	4.40	66
131	47 × 53	+2.30	7	−2	6	0	0	4	22	28	0	54	4.44	65
87	29 × 32	+3.25	9	−3		0	0	0	28	25	0	53	4.47	71
146	48 × 58	+4.75	7	2		0	0	7	8	12	7	34	4.56	64
152	49 × 56	+9.25	12	6		0	0	10	14	1	18	43	4.63	68
136	47 × 58	+6.70	15	0		0	0	5	7	10	9	31	4.74	65
149	49 × 51	+3.50	8	−7	2	0	0	7	15	9	18	49	4.78	65
151	49 × 55	+6.55	11	4		0	0	3	5	5	11	24	5.00	67
153	49 × 57	+.15	5	−5		0	0	3	5	42	14	64	5.05	68
	Average	4.36	8.7	−0.4									4.58	66.7

[2] 9 rows.

The number of hybrids at the two ends of the range of maturity is not sufficient to indicate whether this is actually the case. Such a curvilinear relationship, if it exists, could be due perhaps to the approach to a peak of moth oviposition at the beginning and another at the end of the range of flowering. An examination of table 3 will indicate that it is possible to pick out hybrids each with low damage but with a considerable difference in the dates of flowering. Thus hybrids with the lowest and third from the lowest average class of injury were evidently of approximately the same stage of maturity as were the hybrids with the third and fourth from the highest average class of injury.

In 1931 a study was also made of 149 hybrids involving more than 50 different inbreds selected from Pride of Saline. These showed a range in average class of injury from 2.36 to 5.05 and a range in maturity as measured by days from planting to one-eighth pollen shedding of from 63 to 74 days. A study of the scatter diagram of this group of hybrids indicates little more relationship between maturity and average class of injury than was shown by the early hybrids mentioned above, nor is there any evidence of curvilinear relationships. The data from the 12 highest and 12 lowest in earworm injury are shown in table 3. A study of the hybrids with the lowest and the highest average class of injury gives further evidence of the lack of relationship between these two variables. For instance, the hybrid with the highest and the one with the lowest average class of injury were identical in time of shedding pollen and probably in time of silk emergence.

Among these Pride of Saline hybrids the relation between class of injury and the days to pollen shedding was studied in the 51 constituent inbreds, the data being based on the average performance of inbreds in from 2 to 10 single crosses. The average days from planting to pollen shedding varied from 64.0 to 72.8. On this basis of performance of inbreds in crosses there again appeared to be no close relationship between average class of injury and days to one-eighth pollen shedding. This is further indicated in table 4, in which the parental strains were divided into quartiles and the average of the two variables for the different quartiles given.

TABLE 4.—*Relation of class of injury by corn earworm to (1) days from planting to one-eighth pollen shedding and (2) to husk extension, among 51 Pride of Saline inbreds, lines based on performance in single crosses*

Quartile No.	Parental strains	Average class of injury [1]	Average time from planting to ⅛ pollen shedding	Average husk extension
	Number		*Days*	*Centimeters*
1	13	3.12	66.4	3.57
2	13	3.49	67.3	4.41
3	12	3.73	68.8	3.40
4	13	4.07	67.7	3.61

[1] Arranged in order of average class of injury.

There is a difference of only 1.3 days between the quartile with the lowest class of injury and that with the highest. The third quartile,

moreover, averaged later than the fourth in the date of flowering, indicating no clear-cut trend between these two variables.

This same lack of any obligate relationship between stage of maturity, as measured by pollen shedding or silking, and class of injury has also been found in most of the other years in which it has been studied in sufficient detail. In 1932, in a special test involving some of the Pride of Saline hybrids, the average class of injury in individual hybrids ranged from 1.80 to 3.18 in two replications. The 2 individual hybrids at the extremes of the range of class of injury varied only by 0.5 day in the number of days from planting to one-eighth pollen shedding. All 20 hybrids involved taken as a group, however, show a slight tendency for the later flowering ones to have a higher average class of injury. When the inbreds in this same test were studied on the basis of their performance in single hybrids there appeared to be no close relationship between this stage of maturity and class of injury. For instance, inbred parent 14, represented in 10 hybrids, and inbred parent 30, represented in 8 hybrids, showed a difference in class of injury of 0.52 ± 0.1004, which is statistically highly significant. These same parents as measured by their performance in hybrids differed by 1.3 days in respect to this stage of maturity. This small difference in dates of flowering can hardly be considered to be of biological significance.

In 1937, 35 three- and four-way crosses and top crosses varied in class of injury from 3.04 to 3.95 as an average of four replications for each hybrid involved. An analysis of variance indicates that the difference in earworm injury between the hybrids is significant. These hybrids ranged in average days from planting to one-eighth pollen shedding from 69 to 77 days, and in days from planting until one half the plants were in silk, from 74 to 84 days. A scatter diagram shows a relationship between both these variables and the average class of injury. Those hybrids with the earlier pollen shedding or silking tend to have the higher class of injury. In one experiment concerned with 10 of these hybrids the correlations of time of flowering with class of injury were significant (with days from planting to one-eighth pollen shedding, $r = -0.69$; with days to one-half silking, $r = -0.68$). In another experiment in which there were 20 hybrids the correlations were still negative but not significant (with days from planting to one-eighth shedding, $r = -0.29$; with days to one-half silking, $r = -0.049$). This trend is exactly the reverse of that found in most years, and, incidentally, is the reverse of that found in a group of popcorn varieties which were studied the same year and which were silking and shedding pollen at approximately the same time. In spite of this reversal of the relationship between class of injury and stage of ear maturity, certain hybrids which had shown a low average class of injury in other years also showed a low average class of injury in 1937. Thus the resistant strains do not appear to be as greatly influenced by date of silking as the susceptible ones. This fact would indicate that it may be the susceptible individuals within a varietal population, or the susceptible strains within a group of hybrids, that are primarily responsible for any general relationship that may exist between injury by earworm and the period of flowering.

Relation of Class Ear Injury to Length of Husk Extension

The length of the husk extension beyond the ear tip was studied on the group of early single crosses in 1931, as shown in table 3. Each hybrid appeared to be reasonably uniform for length of husk, although marked differences in husk covering were evident between hybrids. There was a maximum difference of 12 cm. between the average of hybrids with the least husk extension and those with the greatest. Negative readings indicate ears whose tips protruded beyond the ends of the husks. The correlation between the average class of injury and the average husk extension was negative and highly significant statistically ($r = -0.29$). A study of those hybrids having the least and those having the most injury (table 3) shows that there are 2 among the 12 hybrids with the lowest average class of injury in which the tip of the ear extends beyond the husk, on the average, and that some of the hybrids in the group with the highest class of injury have husks which are as long as or longer than the hybrid with the lowest amount of damage. This would appear to indicate that while the length of husk is a factor among some hybrids it is not the only factor involved and in the case of some hybrids is perhaps of negligible importance.

The same year (1931) the husk extension was measured on the 149 Pride of Saline hybrids listed in part in table 3. In most of these hybrids the husk extended beyond the ear. There was a range in husk extension from an average of +11.15 cm. to −3.0 cm. among the different hybrids represented. In this case there appears to be some relationship between husk extension and class of injury, but this certainly is not an obligate relationship since the average husk extension of the hybrid with the least injury was +6.65 and that of the hybrid with the second highest class of injury was +6.55. Similar comparisons were possible among the other hybrids. When these hybrids are studied on the basis of the constituent inbreds as summarized in table 4 there is only 0.04 cm. difference between the average length of husk extension of the 13 parental strains with the lowest class of injury and that of the 13 parental strains with the highest class of injury. Detailed measurements were not taken in succeeding years in view of the apparent small obligate importance of the character of husk extension within the range available for study among the hybrids here.

Performance of Inbreds in Hybrid Combination Under Various Seasonal Conditions as Measured by Class of Ear Injury

A group of Pride of Saline hybrids has been studied through a period of years and the results have been analyzed on the basis of constituent inbreds. A summary of this analysis is shown in table 5.

TABLE 5.—*Corn earworm injury rating* [1] *of inbred lines from Pride of Saline based on their performance in single crosses, 1930–38*

Inbred No.	1930			1931			1932			1933			1938			3-year average class of injury	4-year average class of injury	Total replications 1931–38
	Plants infested	Rank	Hybrids	Average class of injury	Rank	Hybrids	Average class of injury	Rank	Hybrids	Average class of injury	Rank	Hybrids	Average class of injury	Rank	Hybrids			
	Percent		*Number*			*Number*			*Number*			*Number*			*Number*			*Number*
5	85.3	2	6	2.82	1	5	2.12	2	6				2.52	3	5	2.49		16
4	88.3	4	8	3.28	8	2	2.16	4	2	2.19	8	1	2.19	1	1	2.54	2.46	6
17	91.9	7	3	3.08	5	3				2.15	4	2	2.40	2	4	2.54		9
14	79.6	1	8	2.99	2	6	2.15	3	10	2.21	9	5	2.68	4	8	2.61	2.51	29
11	89.1	5	7	3.01	4	5	2.18	5	4	2.14	3	1	3.01	7	3	2.72	2.59	13
18	93.3	8	8	3.00	3	3				2.29	10	2	2.93	6	7	2.74		12
50	98.2	13	7	3.71	14	4	2.43	9	11	2.15	4	2				2.76		17
19	87.0	3	5	3.16	6	3	2.07	1	4				2.86	5	4	2.79		11
22	100.0	18	10	3.46	10	9	2.43	8	8	2.59	13	2				2.82		19
48	96.7	10	9	3.49	12	9				2.09	2	1	3.21	11	5	2.93		15
30	100.0	18	2	4.14	19	2	2.66	10	8	2.00	1	1				2.93		11
60	95.1	9	4	3.27	7	3	2.36	7	16	2.90	16	1	3.21	11	4	2.94	2.93	24
41	89.9	6	9	3.42	9	7				2.16	7	2	3.29	13	8	2.96		17
37	98.5	15	6	3.69	13	4	2.33	6	7	2.88	15	2				2.97		13
44	97.8	11	6	3.84	16	6				2.37	12	1	3.02	8	7	3.08		14
36	98.4	14	8	3.46	10	8				2.78	14	2	3.08	9	4	3.11		14
39	99.1	17	8	3.85	17	6				2.15	4	4	3.60	15	2	3.20		12
Pride of Saline	90.5			3.70		9				2.41		9	3.54		1	3.22		19
26	98.5	15	9	3.73	15	8				2.90	16	1	3.13	10	8	3.25		17
55	97.9	12	10	4.10	18	10				2.34	11	4	3.32	14	17	3.25		31

[1] In rating injury, 1 denotes ears with no injury.

A study of these hybrids was begun in 1930, in which year only the percentage of plants infested was recorded; in later years damage was recorded on the basis of class of injury. This is the same group that has furnished much of the information given above. In 1930 and 1931 records were taken on the hybrids made up from a group of 51 inbreds. Notwithstanding the fact that the 1930 data were taken on percentage of ears injured and the 1931 data on average class of injury, there is a highly significant interannual correlation between the performance of these 51 inbreds ($r=+0.53$). Twenty-three of these inbreds were represented by hybrids in 1938 and there is again a highly significant interannual correlation in the performance of the two groups ($r=+0.75$).

In table 5, 19 of these inbreds which were represented by hybrids recorded in 4 or more years are ranked on the basis of a 3-year average class of injury. The 3-year average is made up of from 5 to 29 hybrids and each average is based on from 200 to more than 1,000 ears. The average must be studied in the light of the number of hybrids involved in the different years. Inbreds 50 and 48 and probably 30 have too high a rating in table 5 because of the particular years and the number of replications involved in the average. For the other inbreds the ranking is relatively similar in the different tests despite wide seasonal differences and differences in the method of recording injury in one of the years. It appears, therefore, that the differences represented in the table by those inbreds with high and low class of injury are genetic and hence of importance in a breeding program. Corroborative evidence has been furnished by the use of a certain single cross in hybrids involving resistant inbreds in double and three-way crosses. Single cross 4 × 14 proved to be especially useful. During the course of the experimental work a number of inbreds have been discarded, particularly those transmitting extreme susceptibility to corn earworm and other undesirable traits. Hence, it is probable that in the later years the spread between susceptible and resistant hybrids would have been greater had more of the susceptible types been retained.

INBREDS AND HYBRIDS WITH REFERENCE TO BUDWORM INJURY

Each year the corn ear worm does some damage at Manhattan to the upper leaves of young corn plants. This type of injury to the curl has been known as budworm or ragworm injury. On three occasions the damage in the breeding and testing nursery has been so severe that certain rows in which no plants were injured were very conspicuous. This called attention to the possibility that susceptibility to this type of damage might be inherited. In 1929 records were taken, and a range up to 50 percent of the injured plants was found. Since this type of damage does not occur with equal intensity each year there was no opportunity to restudy the strains on which records were taken. In 1934 a heavy budworm infestation occurred, and records were taken on most of the hybrids and inbreds in the agronomy nursery. One group of single-cross hybrids being tested for the first time was of especial interest on account of the wide range in the amount of damage in various plots. All of the corn in these nurseries was destroyed by drought and most of it cut early for forage; hence the same hybrids were replanted the following year with but little change in the number of strains represented. Thus in 1935 when a second budworm in-

festation of similar intensity occurred it was possible to get a second record on identical material. The plants in the several strains were fairly closely related and did not differ materially in height at the time of the infestation. At harvesttime in 1935 it was possible to take records on the class of injury of ears from the same plants previously recorded for budworm injury. The injury to ears, however, showed wide differences according to location in the field, and it was necessary to correct the figures for place effect on the basis of uniform checks which occurred every 10 rows. The early spring infestation in the curl of the plant showed no such variability. The performance of each inbred was calculated on the basis of the performance in hybrids in which it occurred, and the data were averaged for percentage of plants infested in the bud in respective years and average class of ears injured. In 1934, data were taken on the percentage of plants showing curl injury among the actual inbred parents. The number of plants concerned in this figure varied from 16 to 135. A summary of the results obtained is given in table 6.

TABLE 6.—*Relation of curl injury in inbred lines and hybrids to injury of ears in hybrids by the corn earworm—New Yellow hybrids, 1934–35*

Inbred line No.	Inbred line		Performance in hybrids 1934		Performance in hybrids 1935			
	Plants	Infested	Crosses represented	Average plants infested	Crosses represented	Average plants infested—curl injury	Average class of ear injury	
							Observed	Corrected for place effect
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*		
63	60	14	9	5	9	11	3.8	3.6
57	22	0	12	5	12	23	3.3	3.1
25	46	7	6	9	6	21	4.1	3.9
33	31	0	10	11	11	15	3.7	3.5
48	109	3	14	11	14	17	2.8	3.1
51	48	5	27	11	27	18	3.3	3.3
67	34	34	14	12	14	20	3.4	3.6
30	16	51	13	12	13	20	4.0	3.6
53	32	12	21	13	20	19	3.7	3.7
60	84	24	7	13	7	19	3.4	3.2
68	84	19	6	13	6	25	3.5	3.9
70	31	4	5	14	4	18	4.2	3.8
54	31	18	7	14	7	20	4.1	3.9
66	82	55	10	15	10	27	3.7	3.6
45	74	38	8	15	8	35	2.9	3.4
26	76	6	19	18	18	24	3.5	3.4
55	65	52	12	21	11	36	3.1	3.2
56	58	22	24	21	24	37	3.6	3.4
75	45	70	18	24	17	24	3.9	3.8
58	33	17	8	25	7	33	3.3	3.3
64	16	75	5	26	5	37	3.7	3.8
61	135	66	15	27	15	32	3.3	3.3
28	52	25	7	29	7	31	3.2	3.5
73	45	18	4	33	4	37	4.2	3.9
59	56	45	8	35	8	31	3.2	3.2
50	43	68	9	50	9	40	3.8	3.8
42	18	73	9	50	9	30	2.5	3.3

An examination was made of the relationship between the percentage of inbred plants infested and the average percentage of plants infested among the hybrids in which each inbred was represented. The correlation between the curl injury to parents and to F_1 hybrids in 1934 was highly significant ($r=+0.67$). The interannual correlation between the percentage of infested plants in identical hybrids in

1934 and 1935 was also highly significant ($r=+0.73$). On the basis of performance of inbreds it was possible to compare the percentage of plants showing budworm injury with the average class of ears injured in 1935 on hybrids from these same inbred lines. Among hybrids representing 27 inbred lines the correlation between these two kinds of injury was not significant ($r=+0.003$).

These data indicate that resistance to budworm injury, whatever its cause, is inherited, and in some cases, at least behaves as a dominant character. The lack of close relationship between average class of ear injury and percentage of plants showing budworm injury indicates either that these two characteristics are governed by different sets of genetic factors or that the difference in ear injury which results from difference in resistance was distorted so by the date of silking that relationship to budworn injury is obscured. In view of the results reported elsewhere in this paper on class of ear injury, it is believed that the former explanation is the correct one. An inbred which confers on it hybrids a high degree of resistance to budworm injury would be desirable since such a character might tend to limit the increase of the earworm in the field.

MASS SELECTION FOR EARWORM RESISTANCE

In 1932 a beginning was made in a mass-selection experiment for altering earworm resistance within the open-pollinated variety Pride of Saline. Two lots of seed selected in that year, consisting of a group of uninfested ears and a group of fairly severely injured ears, were planted in neighboring areas on the agronomy farm in 1933. Five samples of approximately 50 ears each from consecutive stalks were selected the following fall from distributed areas within each plot. The ears from the uninfested seed corn averaged 0.2 of a class lower than the ears from the infested seed corn. This difference probably is not statistically significant, but an examination of the data indicates that the average of every sample from the plot with infested seed corn showed more injury than the average of the entire other group, and four of the five samples from the plot in which the seed corn was free from infestation showed less injury than the average of the other group. Hence, in this first year the mass selection had given slight but apparently consistent improvement in the expected direction. Ears similar to those selected the preceding year were picked from the two plots and planted in 1934. That year both plots were entirely destroyed by drought and the seed supply was lost. It appears, however, that mass selection may not only provide a promising method for improving open-pollinated varieties for insect resistance, but may furnish a source of breeding material for earworm-resistant inbreds.

DISCUSSION AND CONCLUSIONS

In the material studied, the relationship between date of flowering and earworm damage to ears was much more apparent among varieties than among inbreds and hybrids. Under conditions of extreme infestation such as occur at Manhattan, this relationship is probably due to the greater variability in individual plants of open-pollinated varieties which tends to obscure any resistance that may be present in the general population. The influence of date of flowering on ear-

270958—40——2

worm injury would be most evident in those strains or individual plants that lack other mechanisms of resistance, such as those listed earlier in the paper. It is the predominance of these more susceptible individuals within a variety which is probably responsible for the relationship mentioned. Under other environments with lower incidence of the corn earworm it is possible that the longer silking period of varieties as compared with hybrids would place varieties at a disadvantage on the basis of percentage of infestation.

Among the hybrids or inbreds studied there have been varying numbers of the more susceptible types in the different experiments, and this fact would account for a part of the inconsistency in the correlation between injury and time of flowering in the various experimental groups. An examination of table 3 will furnish additional information on some of these points. In the experiments there recorded, as in other experiments, the hybrids having a low class of injury are generally less variable in respect to this character than are hybrids with a higher class of injury. The ears of the less-injured strains are classified most frequently in two or three classes, whereas those of the more severely injured strains fall into four or five classes; less commonly into three or six. The greater variability of the latter hybrids is perhaps a result of their sensitiveness to environmental influences because of higher susceptibility. These conclusions are based on conditions of high infestation at Manhattan and might not hold under different conditions or in tests where damage was recorded as a percentage of infested ears.

Another evident reason lies in the seasonal variability in the peaks of moth oviposition. Unfortunately no detailed information is available on the seasonal life history of the insect during the years in which these observations were made. The years concerned have differed greatly in climatic factors, and unquestionably these have had a differential effect on insect and plant life histories.

The length of the husk extension would have a greater influence on earworm damage among strains which are susceptible in other respects than among those which are more resistant. This may explain the correlation between husk extension and ear damage in some cases, but at the same time it is equally evident that long husks are not responsible for low damage in some of the hybrids. It appears possible, therefore, to obtain some degree of resistance to earworm injury independent of both husk extension and period of flowering. From time to time observations have been made on tightness of husk and other characters which might be concerned in earworm injury. So far no promising or easily measured characters have been found.

The most important result of these investigations is the evidence obtained that certain inbreds as represented by their performance in hybrids are consistently less injured than others. Year after year, in spite of considerable differences in seasons and in intensity of infestation, there appears to be evidence of the inheritance of resistance independent of some of the measurable mechanisms and of fluctuations caused by the environment. The differences in earworm damage, although small, may be of importance in their promise of future possibilities in specific selection for earworm resistance. The hybrids studied have been from inbreds selected for characters other than earworm resistance. Such resistance as has been found has resulted from random selection insofar as earworm damage is concerned.

Since differences exist under these circumstances it is probable that conscious selection for earworm resistance would yield greater differences. It appears worth while to utilize such differences as are at present available in commercially desirable hybrids. Corn breeders should not lose sight of the possibility of including in their inbreds and hybrids the best qualities of insect resistance available in the germ plasm of the foundation varieties.

SUMMARY

A consistent tendency toward resistance or susceptibility to earworm damage to ears has been transmitted by certain inbred lines of corn.

Length of husk extension and date of flowering have some influence on the amount of damage to ears, particularly in heterogeneous material, but many marked breaks in the correlations occur, suggesting other and more subtle causes of differences in severity of injury.

Under conditions at Manhattan, Kans., the relatively susceptible strains appear more sensitive to the influence of date of flowering and length of husk extension than do the relatively resistant strains. The greater influence of these factors in open-pollinated varieties may be due to the high proportion of susceptible individuals present in the material studied.

Differences in resistance and susceptibility to injury to the developing curl or bud of young corn plants are also inherited. Such differences appear to be independent of the differences in resistance to damage to ears caused by the same insect.

There are indications that resistance to earworm injury may be increased by mass selection within an open-pollinated variety.

LITERATURE CITED

(1) Barber, George W.
1936. The cannabalistic habits of the corn ear worm. U. S. Dept. Agr. Tech. Bul. 499, 19 pp., illus.

(2) Burk, Earl F., Cross, C. B., and Hixson, Ephriam.
1936. Variety tests of sweet corn and its resistance to corn ear worm and smut injury. Amer. Soc. Hort. Sci. Proc. (1935) 33 (Ann. Mtg. 32): 502–504.

(3) Cartwright, O. L.
1930. The rice weevil and associated insects in relation to shuck lengths and corn varieties. S. C. Agr. Expt. Sta. Bul. 266, 28 pp., illus.

(4) Collins, G. N., and Kempton, J. H.
1917. Breeding sweet corn resistant to the corn ear worm. Jour. Agr. Res. 11: 549–572.

(5) Ditman, L. P., and Cory, E. N.
1933. The response of corn earworm moths to various sugar solutions. Jour. Econ. Ent. 26: 109–115, illus.

(6) ——— and Cory, E. N.
1936. The corn earworm. Md. Agr. Expt. Sta. Bul. 399 pp. 77–90, illus.

(7) Dungan, G. H., Woodworth, C. M., Lang, A. L., Bigger, J. H., and Snelling, R. O.
1938. Developments in hybrid corn production. Ill. Farmer's Inst. Rpt., 51 pp., illus. Springfield, Ill.,

(8) Hawthorn, L. R., and Fletcher, R. K.
1934. Earworm damage in sweet corn varieties. Amer. Soc. Hort. Sci. Proc. (1933) 30: 550–553.

(9) HINDS, W. E.
1914. REDUCING INSECT INJURY TO STORED CORN. Jour. Econ. Ent. 7: 203–211.
(10) ISELY, DWIGHT.
1935. RELATION OF HOSTS TO ABUNDANCE OF COTTON BOLLWORM. Ark. Agr. Expt. Sta. Bul. 320, 30 pp., illus.
(11) Kyle, C. H.
1918. HUSK PROTECTION FOR EAR CORN. U. S. Dept. Agr. Bul. 708, 16 pp., illus.
(12) MCCOLLOCH, JAMES W.
1920. A STUDY OF THE OVIPOSITION OF THE CORN EARWORM WITH RELATION TO CERTAIN PHASES OF THE LIFE ECONOMY AND MEASURES OF CONTROL. Jour. Econ. Ent. 13: 242–255, illus.
(13) ———.
1922. THE ATTRACTION OF CHLORIDEA OBSOLETA FABR. TO THE CORN PLANT. Jour. Econ. Ent. 15: 333–339.
(14) ———.
1924. THE RESISTANCE OF PLANTS TO INSECT INJURY. Kans. State Hort. Soc. Bien. Rpt. 37: 196–208.
(15) MANGELSDORF, P. C.
1934. GROWING SWEET CORN IN TEXAS. Tex. Agr. Expt. Sta. Cir. 69, 27 pp.; illus.
(16) PHILLIPS, W. J., and BARBER, GEORGE W.
1931. THE VALUE OF HUSK PROTECTION TO CORN EARS IN LIMITING CORN EARWORM INJURY. Va. Agr. Expt. Sta. Bul. 43, 24 pp., illus.
(17) ——— and BARBER, GEORGE W.
1936. OVIPOSITION BY HELIOTHIS OBSOLETA FAB. ON THE SILKS OF CORN. Va. Agr. Expt. Sta. Tech. Bul. 58, 14 pp. illus.
(18) POOLE, CHARLES F.
1935. CORN-EARWORM RESISTANCE IN MAIZE VARIETIES AT DAVIS, CALIFORNIA, 1934. Amer. Soc. Hort. Sci. Proc. (1934) 32 (Ann. Mtg. 31): 453–457.
(19) ———.
1936. NEW SWEET CORNS RESISTANT TO EARWORM. Amer. Soc. Hort. Sci. Proc. (1935) 33 (Ann. Mtg. 32): 496–501.
(20) SNEDECOR, GEORGE W.
1938. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Rev. ed., 388 pp., illus. Ames, Iowa.

PHOTOTHERMAL INDUCTION OF FLOWERING IN SUGAR BEETS[1]

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INTRODUCTION

Induction of flowering in sugar beets (*Beta vulgaris* L.) has two important practical aspects. Development of seedstalks, called bolting, is objectionable when the crop is grown for sugar production, but when the crop is grown for seed production initiation and complete development of seedstalks in all the plants is desirable. The first curly top-resistant varieties were easy-bolting types (*4*).[3] Their bolting tendency was sometimes objectionable in plantings for sugar, but it was beneficial in plantings for seed. Therefore seed of these varieties could be produced abundantly in relatively mild climates by planting in early fall and allowing the plants to grow in the field through the winter (*21*). Breeding to combine nonbolting with curly top resistance was started as soon as it became evident that there were objections to the easy-bolting tendency of the first curly top-resistant varieties. Nonbolting varieties with curly top resistance were promptly developed; but, when the effort was made to produce seed by the method of overwintering in the field in the relatively mild climates where beet-seed growing had become established, these varieties failed to reproduce satisfactorily (*22*). Nonbolting varieties bred in northern Europe gave similar results.

These experiences emphasized the need for fuller knowledge of the principles concerned in bolting and sexual reproduction. This paper presents results of studies of some of the physiological and genetic aspects of the problem.

REVIEW OF LITERATURE AND DEFINITION OF TERMS USED

In much that has been written about causes of flowering, the combined effect of temperature and day length, or photoperiod, which is required by some plants, has not been considered. This is especially true in much of the literature on photoperiodism that has appeared since the classic discovery of Garner and Allard (*9*). The specific effect of temperature has been given prominent attention, however, in extensive Russian work that began with the treatment of seed to induce flowering. The action of temperature has been referred to (*12*) as thermal induction and the influence of day length as photoperiodic induction. Chroboczek (*5*) and Steinberg and Garner (*24*) showed that both low-temperature treatment and long photoperiods are

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[2] The Curly Top Resistance Breeding Committee cooperated in the work in northern Utah and southern Idaho and in the bolting test at Riverside, Calif. This committee represents all the beet-sugar companies in the Western States. Land was furnished for the bolting test at Riverside by the California Agricultural Experiment Station.

[3] Italic numbers in parentheses refer to Literature Cited, p. 123.

favorable to flowering in beets. Roberts and Struckmeyer (*23*) reported important associations between temperature and photoperiod in relation to flowering in a number of species, including beets. The effect of temperature and photoperiod on beets will be considered further in this paper.

Induction of flowering under the influence of prolonged low temperature and long photoperiods may be tentatively regarded as a single process and may therefore be designated by a single term. The term "vernalization" has sometimes been used for this purpose, including induction of flowering as it occurs in nature and under artificial conditions, but this term has been applied mostly to thermal induction (*16*) and generally to hastening of flowering by prolonged low-temperature treatment of germinating seed. Furthermore, "vernalization" has also been used (*7, 25, 27*) to include beet-seed treatments involving germination at higher temperatures, even though such treatments do not induce flowering. . The term "vernalization" will be used in this paper to mean prolonged low-temperature treatment of seed during germination to hasten flowering. For the broader meaning, including thermal induction and photoperiodic induction, the new term "photothermal induction" [4] will be used. Photothermal induction occurs in beets (*5, 24*), celery (*26*), some wheats (*17*), and many other species.

Photothermal induction of flowering in beets is influenced by genetic factors. Munerati (*20*) investigated an annual beet and showed that a single genetic factor was associated with a clear-cut annual habit. Working with this annual strain obtained from Munerati, Abegg (*1*) demonstrated linkage between the bolting factor *B* and the factor *R* for red hypocotyl color described by Keller (*13*). These findings were helpful in the present study.

"Bolting" as used in this paper is the appearance of the seedstalk or the appearance and development of the seedstalk whether or not flowering is involved.

SCOPE OF STUDIES AND EXPERIMENTAL METHODS

Extensive field observations have been made on commercial and experimental sugar-beet-seed fields in the relatively mild climates of southern Utah, southern California, southern New Mexico, and southern Arizona, and also in the colder climates of northern Utah and southern Idaho, where there is usually some winter snow cover. These observations were started in 1930. More recently, similar observations have been made in Oregon and northern California. All these studies were on plantings made in late summer or early fall and were conducted by the method of overwintering the plants in the field (*21*).

Field observations regarding the factors involved in induction of flowering have also been made on commercial fields of beets grown for sugar production in California and other Western States.

Intensive bolting studies were conducted in field plots at Riverside, Calif., in the years 1935–36, 1936–37, and 1937–38. Shading experiments to modify the natural environment were included in these studies.

Greenhouse studies were made with variations in both temperature and photoperiod. An idea of the temperatures used is given by

[4] This term was suggested to the writers by Dr. E. J. Kraus, chairman, Department of Botany, University of Chicago.

showing means obtained by averaging the daily minimum and maximum temperatures. (See table 6.) In interpreting these averages it should be borne in mind that daily maximum temperatures were frequently reached as peaks caused by brief periods of sunshine before ventilators could be opened. Minimum temperatures were reached much more gradually. In general, comparisons were made between rather widely separated temperature levels where precise control was not essential.

Photoperiods were controlled by using artificial light to supplement the natural day length. Mazda globes with 150- to 500-watt capacity were placed over the plants at distances to give intensities of 10 to 100 foot-candles. The photoperiod most commonly used was between 17 and 18 hours. An 8-hour day and a 24-hour day were also used in a few experiments.

Storage experiments were conducted in darkness with plants of intermediate size, usually with a root diameter of 1 to 3 inches. Usually the storage temperature was maintained between 33° and 36° F., with a few tests at approximately 40°.

Seed was given vernalization treatment in cold storage. In most cases the seed lots were first disinfected with a 10-percent solution of commercial formalin for 10 minutes, then washed and soaked overnight and incubated at room temperature under high humidity until the radicles began to emerge. Then in moist condition the seed was stored in closed containers at 33° to 36° F. The germinating seed was allowed to remain at this temperature for various lengths of time, but in most experiments this treatment was continued about 100 days. The sprouted seed was then planted in the field or in the greenhouse.

Material of known genetic constitution with regard to bolting tendency was utilized in some tests. An annual beet obtained from Munerati and previously investigated (*1*, *20*) was used extensively. A few observations were also made with the annual from Milpitas, Calif. (*3*), and also the more extreme annual type from the Imperial Valley of California (*3*). Greenhouse observations were made with several wild species.[5] Vegetative clones were used for the most critical work, and these were propagated and studied over a period of years.

PHOTOTHERMAL INDUCTION IN ANNUAL AND BIENNIAL BEETS

All varieties of *Beta vulgaris* investigated are long-day plants. The chief difference between biennial and annual beets in regard to photothermal induction is the longer period of low-temperature exposure required for flowering in biennials. There is also much variability among biennial types with regard to this requirement, and commercial varieties are made up of a mixture of such types. In biennial beets, photothermal induction is frequently reversed by increasing the temperatures (fig. 1) or by decreasing the length of the photoperiods. This reversal causes the plants to turn vegetative after they have started to develop in the direction of flowering. In annual beets, also, photothermal induction is reversible, but with annuals the influence of photoperiod is stronger than the influence of temperature.

To induce bolting in biennials when grown in the greenhouse with 17- to 18-hour photoperiods it was necessary to hold the average

[5] These wild species were obtained from Dr. G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry.

temperature under about 58° F., although temperatures somewhat higher were effective with continuous light (a 24-hour day), as previously reported by Steinberg and Garner (*24*). All varieties, including annuals, remained vegetative in an 8-hour day under relatively warm temperatures.

Other *Beta* species are also long-day plants. *Beta maritima* L., *B. atriplicifolia* Rouy, the wild or escaped beets of Milpitas, Calif., and the wild beet from the Imperial Valley of California (*3*) are all more or less annual types, but they all remained vegetative in the green-

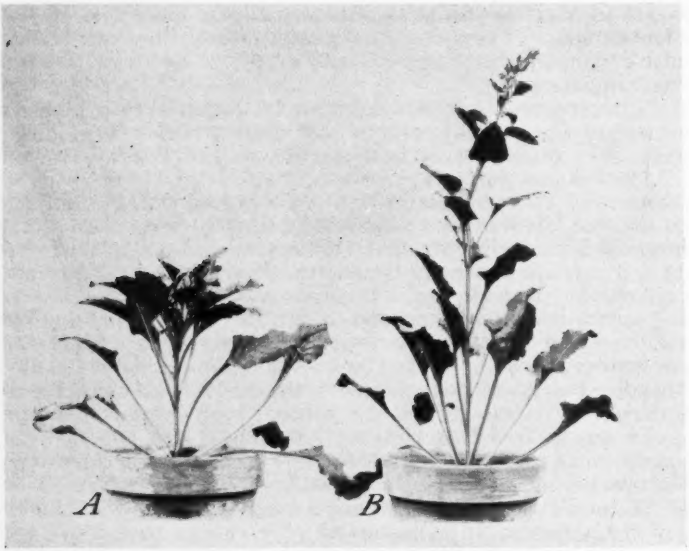

FIGURE 1.—Photothermal induction reversed by changes in temperature. Plants of clone 90 and hence of identical genetic constitution. Seedstalks about 1 inch in height were initiated in both plants under a cool temperature and 17- to 18-hour photoperiod: *A*, Taken to a warm temperature after the initiation of seedstalk, but length of day not changed; *B*, received same treatment as *A* but was returned to the cool temperature after it became somewhat vegetative in the warm temperature.

house under 8-hour days and relatively warm temperatures. The wild species *B. procumbens* Chr. Sm., *B. patellaris* Soland, and *B. webbiana* Moq. developed decumbent branches under both short and long photoperiods, but they failed to produce flowers under the 8-hour day.

The Munerati annual has been investigated (*1*, *20*) more carefully than any other annual variety, and its behavior has been relatively uniform. It is very sensitive to photoperiod, and at Salt Lake City, Utah, in a warm greenhouse (60° to 85° F.) it will not start a seedstalk under natural day length until nearly June 1. It is easily held vegetative in an 8-hour day at any time of the year under a relatively warm temperature. Plants of this variety, even after 30 days' storage at 33° to 36° F., remained completely vegetative under 8-hour days.

For 3 years it has been noted that in October plantings at Riverside, Calif., the Munerati annual remains vegetative all winter and the roots sometimes reach 3 or 4 inches in diameter. Under these conditions its bolting tendency cannot be easily distinguished from that of some commercial sugar-beet varieties. In fact, a portion of the plants in the U. S. 12 variety (*22*) initiated seedstalks somewhat faster during the month of March at Riverside than the Munerati annual.

The influence of temperature on the flowering of annual beets is less obvious than that of photoperiod but under certain conditions can be clearly demonstrated. This opinion is based upon detailed studies with the Munerati annual. Two plants of clone 62, a selection from the Munerati annual, initiated seedstalks in 22 days in a warm room with a 17- to 18-hour photoperiod (average maximum temperature 78.0° and average minimum 54.6° F.). Under the same conditions two other plants from the same clone, previously held at approximately 40° for 39 days, started seedstalks in 15 days, or 7 days sooner than those without the low-temperature treatment. In another experiment with the Munerati annual, two groups of plants were compared that had been grown from seed for 69 days at different temperatures. One group had been grown at a relatively cool temperature (average maximum 74.4° and average minimum 43.5°) and the second group had been grown at a relatively warm temperature (average maximum 80.2° and average minimum 52.9°). On December 2, 1937, after the 69-day treatments at these contrasting temperatures, both groups of plants were placed in the same greenhouse compartment under a 17- to 18-hour photoperiod and relatively cool temperature (average maximum 60.0° and average minimum 43.4°). The plants from the cool temperature, although much smaller than those from the warm temperature, all bolted in 79 days, whereas the plants from the warm room required 92 days for all individuals to bolt. Both experiments show that while seedstalk development in this annual is influenced chiefly by photoperiod, nevertheless low temperature also exerts an influence in the same direction.

A dominant factor *B* is responsible for the annual habit in the Munerati annual (*1*), but the F_1 hybrids with biennial types, heterozygous for *B* and possibly other complementary genes, are slower to bolt than the annual parent. Some of the annual segregates (having the *B* factor) from backcrosses to biennials are very slow to bolt and may easily be confused with biennials under many combinations of temperature and photoperiod. One annual segregate (5357–1), from a backcross to a biennial from a hybrid with the Munerati annual, was propagated vegetatively for 4 years and studied in some detail. It bolted in midsummer temperatures at Salt Lake City, Utah, under the naturally long days occurring at that time. In this way it could be distinguished from biennials, but under relatively cool or intermediate temperatures (40° to 65° F.) several varieties that are considered biennial bolted just as readily. It is evident, then, that the presence of the *B* factor is most easily distinguished by maintaining relatively high temperatures and a long photoperiod.

Some of the wild annuals from Milpitas, Calif., differ from the Munerati annual in that they will bolt in shorter photoperiods. The population of plants from Milpitas is a mixture of genetic types varying in bolting tendency, but most of the plants are annuals. In

spring plantings at Salt Lake City, Utah, they were quite similar to the Munerati annual, but in an October planting at Riverside, Calif., some of the Milpitas plants bolted much faster than the Munerati annual.

On March 12, 1938, at Riverside, Calif., an interesting comparison was available between the Munerati and Milpitas annuals. The planting was made October 6, 1937. In the Munerati annual, seedstalks were just starting to develop on March 12, 1938, and were not over 2 inches in height, whereas the seedstalks of some of the Milpitas plants were over 3 feet in height on the same date and had well-developed flower buds. It is clear that the short photoperiod during the winter at Riverside was responsible for the delayed bolting in the Munerati annual, and the comparison indicates that some of the Milpitas annual plants do not require as long a photoperiod for bolting as does the Munerati annual. Other experiments have shown that the Imperial Valley annual will flower in still shorter photoperiods than the Milpitas annual. At Salt Lake City, Utah, September 9, 1938, seeds of the Munerati annual and Milpitas and Imperial Valley annuals were planted in flats in a warm greenhouse under the natural photoperiod. By November 11 all of the Imperial Valley annual plants had bolted whereas none of the others showed any bolting.

The facts presented show that while bolting in annual beets is induced mainly by long photoperiods, the process is also influenced by low temperature. The evidence also shows distinct differences in bolting tendency between different types of beets. Some are influenced more strongly by photoperiod and others more strongly by low-temperature exposure.

EFFECT OF PROLONGED LOW-TEMPERATURE EXPOSURE

The induction of flowering in beets by prolonged low-temperature exposure has been observed in experimental field plantings at Riverside, Calif., made in September, October, and November. Here the climate is such that the plants grow all winter, and temperatures during December, January, and February are in the main favorable for induction of flowering. The photoperiod during these months is, of course, short. All the varieties of beets, including some annuals, when grown from seed planted in the fall, remained vegetative in these tests until about March 15 or later, and some of the more vegetative varieties never developed more than a trace of bolters. The behavior was different when plants previously stored for a long period at low temperatures were planted. Plants selected June 2, 1937, for nonbolting, from a strain planted as seed on October 13, 1936, were stored at 36° to 38° F. from June 2 to November 4, 1937, and then transplanted in the field. On January 20, 1938, 7 out of 31 of these beets, or 23 percent, had started to bolt and in one case a stalk was 17 inches high. Later in the spring all the plants flowered normally. The same variety planted as seed in the field October 6, 1937, did not bolt until April 1938. Less than half of the plants in this lot bolted, and none flowered completely. Obviously, induction of flowering was strongly influenced in the plants given prolonged low-temperature exposure.

Evidence of a similar nature was observed with the nonbolting variety U. S. 15. The seed was planted in a 5-acre field near River-

side, October 15, 1935, and a part of the field was maintained 2 years. There was less than 1 percent bolting in the spring of 1936, and in 1937 also most of the plants remained vegetative or bolted too late to produce seed. Roots taken from this field were stored at 36° to 38° F. from June 16 to November 1, 1936, and then planted in an experimental plot at Riverside.

On the same date, November 1, stecklings were transplanted directly from the 5-acre field to the experimental plot. By February 5, 1937, seedstalks had started on two of the plants that had been stored at low temperatures. By March 2, several more showed short seedstalks, and on May 11 all 44 of the plants had bolted, and with fair uniformity in that all but one had buds formed or open flowers. Out of 49 plants transplanted in November directly from the field, only 28 produced seedstalks, and these were extremely irregular in development. Here

FIGURE 2.—Short seedstalks produced in an unfavorable environment after a long period of low temperature storage.

again, prolonged low-temperature exposure resulted in earlier and more complete flowering.

Greenhouse studies showed that after prolonged low-temperature treatment stecklings or mother beets may be so strongly inclined toward flowering that they will develop seedstalks a few inches in length in an environment unfavorable to photothermal induction. Beet 138, selected in 1932 and maintained as a clone since that time, was studied in detail in relation to the length of low-temperature treatment required for initiation of the seedstalk. No. 138 is a nonbolting type and remains vegetative under many conditions where most beets develop seedstalks. A 3-month treatment at 33° to 36° F. did not induce bolting in beet 138 except when followed by a favorable bolting environment, a long photoperiod, and cool temperature. In an experiment started June 6, 1935, a supply of thrifty plants grown from cuttings of No. 138 was taken to cold storage and held at 33° to 36°. On November 25, after a treatment for 172 days, these cuttings were removed from cold storage and planted in the greenhouse in two environments. One environment was favorable to bolting, with a relatively cool temperature (40° to 65°) and a photoperiod of 17 to 18 hours, while the other was unfavorable, with a relatively warm tem-

perature (60° to 75°) and with only the natural light of the short winter days.

By January 15, 51 days after planting, seedstalks had appeared on all the plants in both environments. In the favorable environment the plants continued normal reproductive development, whereas in the warm room the seedstalks turned vegetative after making a short growth and before developing any visible signs of flowers. The two most vigorous plants of the seven in the warm room developed seedstalks 7 to 9 inches in height. A comparable experiment with another variety gave similar results, and the short vegetative seedstalks produced in the unfavorable environment are shown in figure 2. It is evident that the prolonged low-temperature treatment not only prepared the plants for bolting but actually induced a reserve of some sort, so that the reproductive tendency continued for a time in spite of an unfavorable environment.

In other experiments prolonged low-temperature treatment was followed by a controlled 8-hour day under warm temperature, making the environment still more unfavorable for bolting. Out of 20 stecklings of U. S. 33 (3407) (*22*) stored 246 days at 33° to 36° F., 3 plants developed 2-inch seedstalks.

EFFECT OF SHADE UNDER FIELD CONDITIONS

Early in the work of breeding sugar beets for curly top resistance, difficulty was encountered in producing seed at Riverside, Calif. Field observations in this mild climate from 1919 to 1928 revealed that better flowering resulted in the colder seasons and that in the warmer seasons, when dormancy in peaches and walnuts was seriously prolonged, flowering in sugar beets was unsatisfactory.

Two simple experiments in regard to the effect of temperature on flowering in sugar beets were conducted at Riverside in the winter of 1928–29. In one case a portion of two short rows planted late in the fall was shaded through the winter months by means of unbleached muslin. The cloth was on a frame that rested on the ground on the south side of the rows and leaned to the north, so that no direct sunlight could reach the beets or the soil in the protected part of the plot. Soil temperatures at a depth of approximately one-half inch in the shaded and in the unshaded soil were recorded by means of thermographs. The records were not taken during the entire period, but the difference between the temperatures in the two environments from January 8 to March 1, a period of 1,248 hours, is indicative of the contrast in conditions to which the shaded plants and those in full sunlight were exposed. During the period mentioned the soil temperature in the shade was continuously below 60° F. During this same period the temperature of the unshaded soil was above 60° for a total of 289 hours, or 23 percent of the total time. Bolting counts on June 4, 1929, revealed that in the unshaded plants 62 of the 347, or 18 percent, had bolted, while in the shaded plants 50 of the 130, or 38 percent, had bolted.

Another test in the same winter involved growing one lot of potted plants in full sunlight and another lot on the north side of a nursery lath house, where the plants were protected from direct sunlight throughout the winter months. Owing to the elevation of the site and consequent air drainage, the temperatures were relatively warm.

Some of the pots in each case were submerged in the soil and others were left on top of the ground. There was only a trace of seedstalk development in the plants exposed to sunlight, while nearly all the shaded plants developed vigorous seedstalks. Soil temperature records were not taken. The temperature differences probably corresponded to those in the previous experiment.

The results of these two tests indicate that in mild climates, such as that of Riverside, Calif., induction of flowering was increased when the beets were kept cooler during the winter by means of shade.

As a result of renewed interest in the factors involved in induction of flowering in beets, a field experiment with artificial shade was started at Riverside, Calif., in the fall of 1936, to get further evidence

FIGURE 3.—Shade in relation to soil temperature and bolting. Horizontal shading frames in foreground; vertical shading frames in background. (Photo graphed November 26, 1937.)

on the influence of temperature. The beets were planted September 18 on single-row beds running east and west. Portions of these rows were then shaded by frames made of shingles coated on both sides with aluminum paint. The frames were placed in series, so that they would shade the beet rows to the north and reflect light on the rows to the south. This vertical type of frame, together with a flat or horizontal type used in a later experiment, is shown in figure 3. There were three different treatments with the vertical frames, as follows: (1) Portions of rows were shaded only during the warmer part of the fall, September 18 to November 12, 1936; (2) other portions of rows were shaded only during the colder part of the season, November 12, 1936, to April 1, 1937; and (3) other portions of rows were shaded from September 18, 1936, to April 1, 1937. Five varieties, all rather low in bolting tendency, were used. There were duplicate plots for each shading treatment. Soil temperatures close to the north side of the beets and at a depth of about one-half inch were

taken in the shaded and unshaded plots by means of soil thermographs. Temperatures of the beet crowns at about one-half inch below the soil surface were determined by means of chemical thermometers inserted snugly into holes made with a cork borer in the sides of the roots. The temperatures of the roots were almost the same as the soil temperature.

Bolting counts on May 10, 1937, gave the following averages: Unshaded, 66 percent; shaded (1) September 18 to November 12, 63 percent, (2) November 12 to April 1, 89 percent, and (3) September 18 to April 1, 79 percent. Temperature records are given in table 1. Shading during the warmer part of the fall did not increase bolting and may have been detrimental in some way. Shading during the colder weather increased bolting.

TABLE 1.—*Temperatures of shaded and unshaded soil at one-half-inch depth and percentage of bolters in varieties 550 and 617 with and without artificial shade*

Treatment	Temperature			Plants bolting [2]
	Daily average [1]	Mean daily deviation		
		Above average	Below average	
	°F.	*Day degrees* [3]	*Day degrees* [3]	*Percent*
Nov. 23, 1936, to Mar. 21, 1937:				
No shade	49.16	5.13	5.22	88
Vertical shade	43.59	3.41	3.60	100
Nov. 22, 1937, to Mar. 20, 1938:				
No shade	51.34	3.09	3.02	47
Vertical shade	50.51	2.40	2.61	79
Horizontal shade	52.17	1.90	2.01	81

[1] Average temperature determined by planimeter measurements of thermograph charts.
[2] Averages of the two varieties.
[3] "Day degree" is defined as a measure of accumulated temperature and represents an average of 1 degree of deviation from the base temperature during a period of 24 hours. Day degrees were measured as areas above and below the base temperature on thermograph charts by means of a planimeter.

Another shading experiment in the field was started at Riverside in the fall of 1937. Vertical frames, of the type used in the previous experiment, and horizontal frames were used. The latter were made of heavy kraft paper coated on both sides with aluminum paint and supported on frames of wood and wire. These frames were 18 inches wide and 12 feet long. The beet rows were 20 inches apart, so that when the frames were laid between the rows nearly all the soil between the rows was shaded (fig. 3). The beets were planted October 6, and the shading was started November 19. Two of the varieties included in the previous year's test, 550 and 617 (U. S. 14), were used in the experiment. These two varieties have practically the same bolting tendency. The shading was discontinued March 21. The results in percentage of bolters and the soil-temperature records are summarized in table 1. The bolting percentages are averages for the two varieties used.

The percentage of bolting was higher in the shaded than in the unshaded plots each season (table 1), and higher in the colder season of 1936–37 than in 1937–38 (table 1).

The fact that in the second season more bolting occurred in the plots with horizontal shades than in the unshaded plots was probably due to the temperature having been lower in the fall and early winter in the shaded plots (fig. 4). The mean temperature for the 7 weeks November 22, 1937, to January 10, 1938, was 1.08° F. higher in the

unshaded plots than in the plots with horizontal shades. The accumulated deviation above 51.19° (the mean for the plots with horizontal shades) was 149.49 day degrees greater in the unshaded plots; the accumulated deviation below 51.19° was practically the same for the shaded and unshaded plots. It is thus evident that during the late fall and early winter the beets under the horizontal shades were in a colder soil environment than were the unshaded beets. Later, as the leaves grew larger and shaded the soil more effectively, the mean soil temperature in the unshaded plots dropped a little lower than in the plots with horizontal shades. However, the soil temperatures in both plots were favorable to thermal induction during most of the latter part of the winter. The fact that the horizontal shades kept the soil temperatures relatively low in late fall and early winter apparently allowed

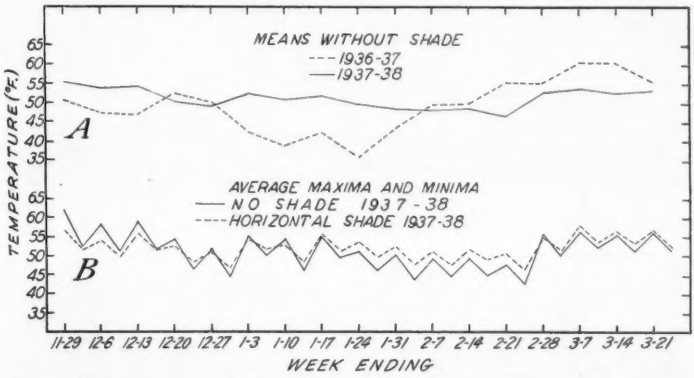

FIGURE 4.—*A*, Mean weekly temperature of soil in beet row at ½-inch depth for the period November 22 to March 21 for the two seasons 1936–37 and 1937–38. *B*, Diagrammatic curves of soil-temperature deviations at depth of one-half inch in beet rows with and without horizontal shades. Curves constructed by using average daily deviation for each week above and below the mean for the same week.

thermal induction to continue longer in the shaded beets, with the result that 81 percent of them bolted, as compared with 47 percent in the unshaded beets (fig. 5).

Many field observations in California and southern Arizona have revealed that with rows running east and west the north sides of two-row beds have more bolters than the south sides. For example, in a field of the low-bolting variety R. & G. Old Type, planted near Calexico, Calif., October 8, 1937, there was a striking difference between bolting on the north and south sides of the beds. On May 15, 1938, there were 37 percent bolters on the north side of the beds and 7 percent on the south side. The temperature of the soil and consequently that of the beet crowns at the same level was often 4° to 5° F. cooler on the north side of the beds. In the field at Calexico the leaf area on the south side of the beds was larger, which gave that side an advantage in regard to induction by light, if it is assumed that leaf area is a factor in this process in beets. It seems probable, therefore, that the greater amount of the bolting on the north side of the beds resulted from induction by temperature.

These observations and experiments gave evidence of the relation of the temperature of beets, as influenced by the temperature of the surrounding soil, to bolting. In mild climates where winter temperatures may be too high for flowering or even for bolting, more bolting and flowering result from a slight lowering of the temperature for a pro-

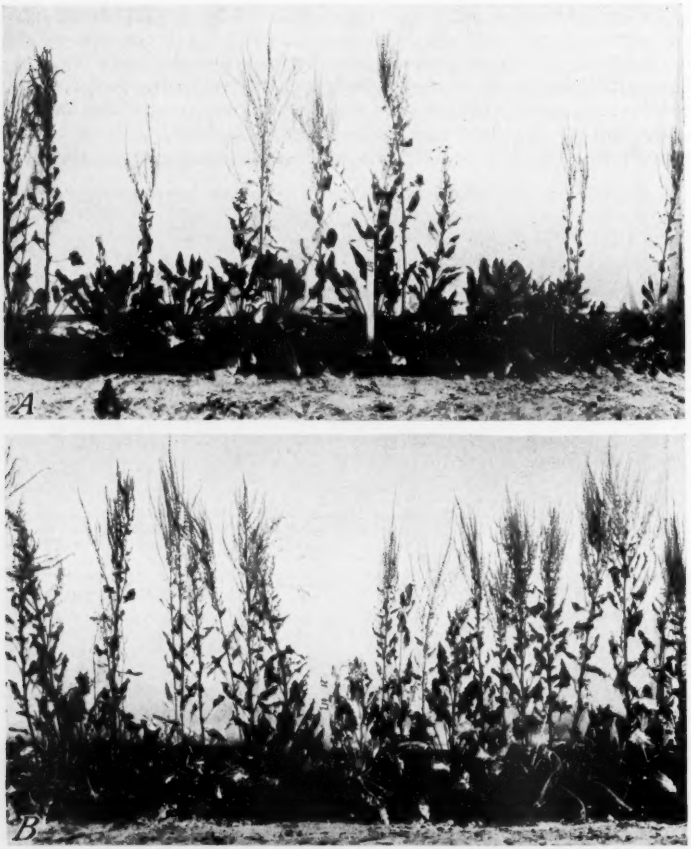

FIGURE 5.—Bolting in shading experiment at Riverside, Calif., with variety U. S. 14, planted October 6, 1937. Photographed June 7, 1938. *A*, Without artificial shade; *B*, soil between rows shaded with horizontal shades of aluminum-painted paper from November 22, 1937, to March 20, 1938.

longed period. Shading the soil surrounding the beets during the winter keeps the beets enough colder to increase bolting.

VERNALIZATION OF SEED AND EFFECTS OF IRREGULAR GERMINATION

Increased bolting in sugar beets as a result of subjecting germinating seed to low-temperature treatment has been previously described

(*7, 12, 25, 27*). Numerous tests by the writers showed increased bolting in plants grown from seed that had been subjected to vernalization treatment, but in some instances no effect was observed. Results of the following experiments explain this apparent inconsistency.

In May plantings at Salt Lake City, Utah, of seed subjected previously to vernalization treatment, little or no increase in bolting occurred. A considerable increase in bolting was observed, however, when a comparison of vernalized and untreated seed was made in an early March planting (table 2). This difference is to be explained by the fact that there was additional thermal induction in the field in the March plantings and this influence added to that accomplished by vernalizing the seed resulted in the effect noted. Seed that had been subjected to vernalization treatment was planted at Riverside, Calif., in October. There was no increase in bolting the following spring. Evidently the relatively warm weather in the fall resulted in enough reversal of the inductive process to overcome the effect of the seed treatment.

TABLE 2.—*Increased bolting from vernalized seed in a field planting made Mar. 16, 1937, at Salt Lake City, Utah*

Variety	Seed treatment	Plants observed	Plants bolting
		Number	*Percent*
U. S. 12 (618)	No treatment	95	1.05
Do	Washed, incubated and dried, but not vernalized	96	5.20
Do	Washed and incubated and then vernalized at 33° to 36° F. for 61 days.	162	12.95
U. S. 14 (617)	do	122	22.20

Striking results with seed vernalization were obtained in the greenhouse under relatively cool temperatures and with a 17- to 18-hour photoperiod when seed subjected to vernalization treatment was planted in comparison with untreated seed. Two strains low in bolting tendency, 2168 and 4919, and the easy-bolting biennials U. S. 12 and 3401 were used. In addition to these varieties, nonvernalized seed of U. S. 1 and the Munerati annual were included in the test. The results are given in detail in table 3. Eighty-two days after planting, untreated seed of U. S. 12 had given rise to 2.8 percent bolters and the vernalized seed had produced 14.3 percent bolters. With 3401, the untreated seed had given rise to no bolters, while the vernalized seed had produced 36.1 percent bolters. At this time there were no bolters in 2168 and 4919, even with the vernalized seed. Vernalization increased the rate of bolting in the easy-bolting biennials, but did not sufficiently affect the two varieties inherently low in bolting tendency to cause them to bolt in the 82-day period.

The relationship of sprouting to vernalization has been noted in several instances. In a series of experiments in 1933 and 1934, where dry seed was planted in moist soil in flats and held at approximately 40° to 45° F., the germination of most varieties was noticeably irregular. After all sprouted seed in some of the flats was killed by drying and freezing, these flats were watered and placed under temperatures favorable for germination and growth of the surviving seed. The seed that had not sprouted during the vernalization treat-

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ment was then found to be unaffected. Similar seed that sprouted during the vernalization treatment was thermally induced to a considerable extent.

TABLE 3.—*Greenhouse test with vernalized seed* [1]

Variety or strain	Plants bolting from—	
	Nonvernalized seed	Vernalized seed
	Percent	*Percent*
2168	0	0
4919	0	0
U. S. 12 (4900)	2.8	14.3
3401	0	36.1
U. S. 1 (9153)	7.5	
Munerati annual (2240)	100.0	

[1] Vernalized seed was incubated a short time at 70° F. and then held at 33° to 36° for 55 days previous to planting. There were 8 replications of each variety and treatment. Each replication consisted of a single row 30 inches long with 20 to 30 plants to the row. Planted Sept. 24, 1934; bolters counted Dec. 15, 1934.

In a field planting at Salt Lake City made March 16, 1937, vernalized seed of U. S. 12 was compared with two kinds of nonvernalized seed (table 2). Lot 1 of the nonvernalized seed was wholly untreated; lot 2 was washed, incubated at 70° F., and finally dried. This treatment of lot 2 hastened germination in the field during the relatively cold weather of early spring and thereby resulted in more thermal induction, and consequently the bolting was increased. However, the vernalized seed gave more than twice as many bolters as did lot 2 of the nonvernalized seed.

In this same field planting, a vernalized lot of seed of the nonbolting variety U. S. 14 gave even more bolters than the vernalized seed lot of U. S. 12 (table 2). It is well known from experience that U. S. 12 is much more likely to bolt in the field than U. S. 14 (*22*). Before planting it was observed that the vernalized seed of U. S. 14 had germinated to a greater extent than the vernalized seed of U. S. 12. This greater germination during the vernalization treatment probably accounts for the fact that plants grown from the vernalized seed of U. S. 14 bolted more than those grown from the vernalized seed of U. S. 12.

In preparing the seed for vernalization treatment, it was first thoroughly soaked and washed and then held at about 70° F. until the majority of the seed balls showed radicles about 1 mm. in length. It was difficult, however, to obtain a uniform degree of germination, and this lack of uniformity in germination was still more noticeable after a 60-day treatment at 33° to 36°. Some radicles were over an inch in length, while other seeds showed no evidence of germination.

The results of an experiment to discover the extent to which sprouting influences thermal induction in seed are given in table 4. Seed previously moistened and then held at 33° to 36° F. for 55 days was sorted into three classes. Class 1 included seed balls with no visible radicles; class 2 those with such radicles as were evidently less than 3 mm. in length; and class 3 those with all radicles developed and all over 3 mm. in length. Bolting counts 82 days after planting revealed approximately twice as many bolters in classes 2 and 3 as in class 1.

The increased bolting from the seed with visible radicles is highly significant (table 4).

Heritable differences complicate the problem of thermal induction in seed. Strain 2168, for example, was found to sprout more rapidly at low temperatures than strain 3401. It might be expected, therefore, that when treated similarly strain 2168 would be more effectively vernalized. Contrary to such expectation, vernalization may not cause any bolting in strain 2168, but it may cause abundant bolting in strain 3401 (table 3). Such results are explained by inherent differences in bolting tendency. Strain 2168 is inherently a nonbolter, while strain 3401 is an easy-bolting strain. If one of two strains of the same bolting tendency germinated more readily than the other at low temperatures, it would be expected to respond more strongly to vernalization.

Vernalization increased the rate and percentage of bolting provided that (1) the seed sprouted during the cold treatment and (2) the vernalized seeds were planted in an environment favorable enough for induction of flowering to continue. Vernalization did not overcome inherent differences in bolting tendency.

TABLE 4.—*Effect of extent of sprouting on the vernalization of seed* [1]

Strain	Plants bolting from seed showing indicated extent of sprouting		
	No radicles visible	One or more radicles visible on each seed ball but less than 3 mm. in length	All radicles visible and over 3 mm. in length
	Percent [2]	*Percent* [2]	*Percent* [2]
3401	25.9	52.2	60.9
3410 (parent of U. S. 12)	28.8	54.3	53.5

[1] Seed all vernalized in the same container, but seed balls separated and classified at the end of the treatment according to the extent of sprouting. Seed planted in rows 30 inches long with about 20 plants per row. Rows spaced 4 inches apart with 5 replications of each seed classification for each variety. Planted Sept. 24, 1934, and bolters counted Dec. 15, 1934.

[2] Standard error of difference due to extent of sprouting when planted=7.9 percent.

A NEW GENETIC FACTOR FOR BOLTING

The action of certain genes in relation to the physiological process responsible for flowering should be known for a better understanding of photothermal induction. The factor B' now to be described has an important role in the process in beets. It is responsible for an easy-bolting tendency but appears to differ from the factor B described by Munerati (*20*) and Abegg (*1*), because plants carrying B' remained vegetative under field conditions, whereas plants carrying B were strictly annual. It will be seen that B' and B are regarded as allelic factors and dominant to the factor b associated with a nonbolting tendency. Linkage discovered between B' and the color factor R (*13*) made the genetic analysis possible. In order to study the factor B', it was necessary to give careful attention to environmental conditions affecting photothermal induction as well as to the genetic material to be investigated.

PARENTAL MATERIAL

The parental material consisted of three clones selected in 1932 and subsequently studied in detail. Their bolting tendencies were therefore well known. Clone 70, *rrB'b*, provided the factor *B'* for bolting, while clone 79, *RRbb*, and clone 90, *rrbb*, were vegetative types. These clones were selected from rather closely bred lines that had been characterized by a fair degree of uniformity.

Clone 70 was selected from2769, a strain obtained from William W. Tracy, Jr. This plant was a fast-bolting biennial. Experience with vegetative cuttings from it, and with seed from its parental strain and its selfed progeny, showed that it was not an annual under field conditions at Salt Lake City, but it was a fast bolter and was easily confused with annuals under relatively low temperatures in the greenhouse. Clone 70 also carried a genetic factor for self-fertility, which distinguished it from most sugar beets.

Clone 79, *RRbb*, was a strongly vegetative or nonbolting type. It was first propagated vegetatively because of interest in its male-sterile character, which is a convenience in hybridization work. The plant was apparently normal in every other respect, but the pollen grains were completely aborted.

Clone 90, *rrbb*, came from Salt Lake City strain 2167, a curly top-resistant strain with a strongly vegetative or nonbolting tendency. An advantage of clone 90 for crossing is its strong self-sterility, for when it is used as a female parent one can be sure of obtaining hybrids without taking the trouble to emasculate.

METHODS

The male-sterile clone 79, *RRbb*, was pollinated from clone 70, *rrB'b*, and several F_1 plants were grown. The F_1 plants segregated for male sterility and self-fertility. F_2 progenies were produced from some of the self-fertile F_1 plants. Backcross progenies were also produced by hybridizing clone 90 (*rrbb*) with pollen from a few of these F_1 plants selected at random from those producing normal pollen.

Respective progenies were tested for bolting by subjecting some of the seed to a vernalization treatment and growing the plants in the greenhouse. The seed was vernalized for 101 days at 33° to 36° F. At planting time most of the seed had sprouted and the radicles were approximately 0.5 to 2 cm. in length. Some seed lots were more uniform in sprouting than others, and some were affected by the development of mold. Seed of the backcross progeny (4615), which gave the most interesting results (table 5), however, was nearly free from mold, and the radicles were more uniform in length than in most of the other progenies. This proved to be a fortunate circumstance, because the uniformity of the vernalized seed when planted is believed to have had an important bearing upon the clear-cut differentiation secured for the bolting types in this progeny (table 5).

The plantings were made in greenhouse beds 30 inches wide with rows 4 inches apart. Progeny 4615 was planted in 15 of these rows, and 250 plants, or about 17 plants per row, grew from uniformly spaced seed. Owing to limited space, plants of other progenies were more closely spaced, with about 34 plants per row. Nonvernalized seed of the respective progenies was planted at the same time for comparison. Artificial light was used to supplement the regular day

length to make approximately a 17-hour day. The temperature was controlled rather roughly, and a record was taken each day from a minimum and maximum thermometer.

TABLE 5.—*Correlation between time of bolting and hypocotyl color from backcross progenies 4615 and 4612*

Days after planting	Bolters		Vegetative plants		Total plants	Cross-overs	
	Red hypocotyl	White hypocotyl	Red hypocotyl	White hypocotyl			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*[1]
SEED OF PROGENY 4615 VERNALIZED							
47	29	104	98	19	250	48	19.2±2.49
105	29	108	98	15	250	44	17.6±2.41
136	71	108	50	14	243	85	35.0±3.06
SEED OF PROGENY 4615 NOT VERNALIZED							
136	5	34	75	48	162	53	32.7±3.69
217	39	65	41	17	162	56	34.6±3.74
235	57	74	19	8	158	65	41.1±3.91
SEED OF PROGENY 4612 VERNALIZED							
164	0	2	87	88	177		
217	17	15	70	75	177	92	52.0
257	73	85	14	5	177	78	44.1

[1] Standard errors of cross-over values from Immer's tables (*11*). Immer's probable errors divided by 0.6745 give the standard errors.

The growing plants were carefully watched, and where possible the temperatures were increased or decreased to produce what was believed to be the optimum temperature to differentiate plants with bolting tendencies from strongly vegetative or nonbolting types. It will be noted in table 6 that temperatures increased slightly in October and again in January, decreased in March and April, and increased again in May. The distinct decrease in temperatures for March and April was the result of manipulation required to bring out more bolting, after a fairly good separation between bolting and vegetative types had been made in the winter months.

TABLE 6.—*Daily greenhouse temperatures, 1934–35*

Month	Temperature		
	Average maximum	Average minimum	Mean
	° *F.*	° *F.*	° *F.*
September 24 to 30	75.1	40.7	57.9
October	81.3	47.0	64.1
November	73.9	47.0	60.5
December	71.8	53.0	62.4
January	75.3	55.5	65.4
February	71.0	53.7	62.3
March	62.4	42.0	52.2
April	72.1	43.0	57.5
May	80.5	46.1	63.3

Bolting records were taken at intervals as bolters appeared, and in most cases the bolters were pulled when counted in order to allow more space for the remaining plants.

EXPERIMENTAL RESULTS

The extreme differences in bolting due to heredity are shown in figure 6. The Munerati annual plants had all started seedstalks within 50 days after planting, whereas the vegetative strain 2167 showed only a small percentage of bolting 200 days after planting. The selfed progeny of clone 70 was between these two extremes but somewhat closer to the early-bolting side. This performance was in agreement with considerable additional evidence that clone 70 represented a rather fast-bolting type.

Although the plants in the selfed progeny of clone 70 were fast bolters there was considerable variability, as shown in figure 6. Some

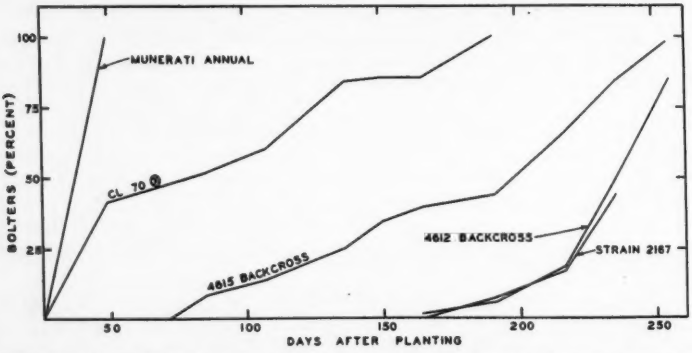

FIGURE 6.—The gradation of beet types from strong annual to strongly vegetative biennial habit is shown in this graph summarizing results obtained with non-vernalized seeds. Clone 70 ⊗ indicates selfed progeny of clone 70. Clone 70 ⊗ and strain 2167 represent parental types, and 4612 and 4615 are backcross progenies from hybrids between clone 70 and clones from strain 2167 described in test.

plants in the progeny bolted much faster than others, but it is uncertain how much of this was due to heredity and how much to environmental differences between different plants. The question of whether or not clone 70 was homozygous for bolting tendency is made clearer by studying its hybrid progenies. Clone 70 is represented by backcross progenies numbered 4612 and 4615 and F_2 progenies numbered 4608 and 4609. The heterozygosity of clone 70 is believed to be shown in the performance of these backcross and F_2 progenies. For the backcross progenies, figure 7 and table 5 illustrate the fact that 4615 segregated for fast-bolting types while 4612 was almost equal to the vegetative parental types. Likewise, the data in table 7 show that the F_2 4608 was fast in bolting while the F_2 4609 was much slower.

It seems probable that clone 70 was of heterozygous constitution, *B'b*, assuming *B'* to be responsible for the bolting tendency. When crossed to clone 79 (*RRbb*) the F_1 plants would be expected to be *RrB'b* and *Rrbb* in equal proportion. Backcrossing the F_1 plants at random to clone 90 (*rrbb*) would then yield some backcross progenies

like 4615, consisting of both *B'b* and *bb* plants that show sharp segregation for bolting and vegetative types, and other backcross progenies like 4612, all *bb*, which lean rather strongly to the vegetative side. Likewise in F_2 progenies, data presented in table 7 indicate that progeny 4608 was derived from a plant of constitution *RrB'b* and progeny 4609 from a plant of constitution *Rrbb*.

LINKAGE BETWEEN *B'* AND *R*

The correlation between bolting tendency and hypocotyl color is a point of much interest. This correlation is shown for the backcross progeny 4615, clone 90, *rrbb*, × (clone 79 × clone 70), *RrB'b*, in figure 7 and table 5. Figure 7 shows, especially with the vernalized seed, than the plants with white hypocotyls (*rrB'b*) bolted much more rapidly that those with red hypocotyls. Evidently because of their genetic constitution, the white hypocotyl plants were in large part so strongly affected by the seed treatment that they bolted quickly after planting. Since the bolting tendency was associated with the

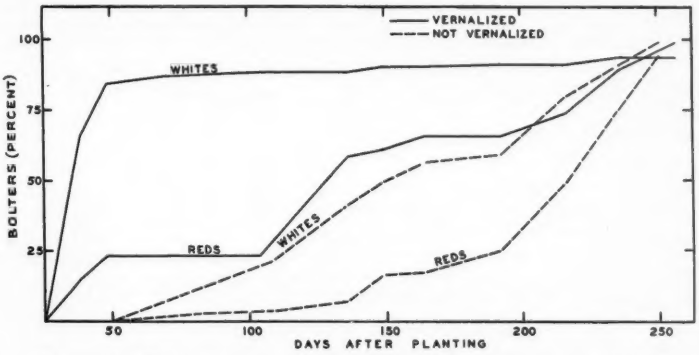

FIGURE 7.—Bolting reaction of color types in backcross progeny 4615.

recessive hypocotyl color in the clone 70 parent, it can be assumed that the respective genetic factors responsible for these characters were linked in their inheritance.

A cross-over value of 15.5 percent between the factor *R* for hypocotyl color and the factor *B* for annual habit has been reported by Abegg (*1*). In table 5, progeny 4615 shows a cross-over value of 17.6 percent between *R* and *B'* at 105 days after planting, which is very close to Abegg's value. Since the rate of bolting is perhaps not entirely clear-cut, even at 105 days after planting, it may be assumed that a more perfect separation between bolting and vegetative types would lead to a slightly lower figure than 17.6 percent crossing over. Calculations from figures obtained from a less desirable classification, made at 136 days after planting, give 35 percent crossing over, which is a poor estimate because of an excess of observed plants in the bolting class over the expected proportion. It is assumed from the similar association of *B'* with *R*, and of *B* with *R*, that *B* and *B'* may be allels with identical positions on the chromosome. There is, however, no critical proof that *B* of the Munerati annual and *B'*

of clone 70 are not the same gene with the apparent difference between them caused by additional modifying factors.

The F_2 progenies were not expected to yield data that would be as clear-cut as those from the backcross progenies. This was particularly true because in the F_1 cross, clone 79 (*RRbb*) × clone 70 (*rrB'b*), the characters entered in the repulsion phase. However, table 7 shows that a significant negative correlation was obtained between bolting and hypocotyl color in F_2 progeny 4608. The χ^2 value has been used to measure this correlation in table 7. This value is highly significant, but it does not give an accurate idea of the percentage of recombinations.

TABLE 7.—*Correlation between time of bolting and hypocotyl color from F_2 progenies 4608 and 4609* [a]

Days after planting	Bolters		Vegetative plants		Total plants	χ^2 [b]
	Red hypocotyl	White hypocotyl	Red hypocotyl	White hypocotyl		
	PROGENY 4608					
	Number	*Number*	*Number*	*Number*	*Number*	
40	77	36	387	89	589	9.461
85	171	75	282	40	568	28.186
172	235	85	162	26	508	11.244
	PROGENY 4609					
39	6	4	282	90	382	
108	69	25	219	69	382	0.266
192	161	53	127	41	382	.007
257	276	90	12	4	382	

[a] All data are from vernalized seed of progenies 4608 and 4609.

[b] According to Fisher's tables (*8*), with 1 degree of freedom χ^2 should exceed 6.635 for the 1-percent point of significance.

DISCUSSION AND CONCLUSIONS

Initiation of seedstalks and flowering of biennial beets is brought about mainly by the cumulative effect of low-temperature exposure followed or accompanied by the effect of long photoperiods. The effect of temperature may be as pronounced as the effect of photoperiod. With beets it is difficult in many cases to distinguish between these two principal environmental effects; hence "photothermal induction" is used in this paper to denote induction of flowering and includes both photoperiodic and thermal induction.

Thermal induction and photoperiodic induction probably result in the production of one or more flower-inducing substances, or hormones, as indicated by work with other plants by Čajlachjan (*2*), Moškov (*19*), Melchers (*18*), Loehwing (*15*), and by one of the writers with beets.[6] Prolonged thermal induction in some cases influences the reproductive tendency so strongly that this tendency continues for some time after the environment has become distinctly unfavorable for reproduction. It appears, therefore, that if induction of flowering is caused by some substance, enough of this substance accumulates while temperatures are favorable to last for some time after conditions become unfavorable.

[6] STOUT, M. Unpublished work.

In the reproductive process the role of low-temperature exposure comes in mainly in the initial stages. In biennial beets somewhat higher temperatures are more favorable for growth of the seedstalks and flowering, as has been reported in the case of tulips (*10*) and spinach (*14*). However, continuation of relatively cool temperature is necessary for completion of flowering in beets. Steinberg and Garner (*24*) reported that in a biennial variety of beets seedstalks were initiated and some flowers produced at 73° F. with continuous light, but the photographs published by these writers show that the seedstalks were somewhat vegetative under this relatively warm temperature. Obviously, the induction was inadequate for complete reproductive development. The writers have observed that vegetative seedstalks similar to those pictured by Steinberg and Garner commonly result from inadequate low-temperature exposure during seedstalk initiation or from excessively high temperatures during seedstalk development.

The relative importance of thermal induction and photoperiodic induction varies with different types of beets according to their genetic constitution. A comparison of the biennial U. S. 12 and the Munerati annual in fall plantings at Riverside, Calif., illustrates this point. In early spring, when the days were relatively short, some individual plants of the biennial U. S. 12 showed seedstalks before any stalks appeared in the Munerati annual. Evidently the U. S. 12 plants were so strongly influenced by thermal induction that they started to develop seedstalks during relatively short days that were unfavorable. The Munerati annual, on the other hand, needs little or no thermal induction, but it requires a relatively long photoperiod. Evidently the Munerati annual requires more photoperiodic induction for initiation of flowering than does U. S. 12 or else does not respond to low-temperature exposure so readily.

The bolting factor *B* (*1*, *20*) is responsible for much of the annual tendency in the Munerati annual. All plants with this factor that have been studied critically at Salt Lake City, Utah, have initiated seedstalks and flowered under long days during midsummer temperatures. A factor *B′* with an effect comparable to that of *B* is described in this paper. Plants with this factor, when grown under long days and at warm temperatures, will flower as readily as the Munerati annual if they have had a short treatment with low temperature.

The influence of the genetic factors *B*, *B′*, and *b*, which determine the tendency for bolting in beets, is comparable in importance to the influence of temperature and photoperiod. Furthermore, the discovery of these genetic factors affords a new approach to the study of induction of flowering. It is now clear that this physiological process would be better understood if more knowledge were available regarding the physiological action of the genes. In the case of *Hyoscyamus niger* L., Melchers (*18*) gives evidence for a gene for biennial tendency with physiological significance somewhat similar to that of *B* or *B′* in beets. Genes in soybeans that influence time of flowering also have been reported (*28*), and this fact should be useful in studying the effect of photoperiod peculiar to this plant.

Reversal or prevention of the induction of flowering is an important factor in vegetative development in beets. This reversal or prevention permits continuation of growth and the accumulation of food reserves.

These food reserves are necessary for beets to survive as biennials, and it is because of the abundant supply of stored food in the form of sucrose that sugar beets are of commercial importance as a source of sugar. Reversal of induction of flowering makes possible the perennial tendency in biennial beets that is evidenced in seed fields where many plants survive and resume vegetative development after the seed is harvested. In contrast to the behavior of biennial beets in this respect, the Munerati annual, after seed has been harvested from it in July at Salt Lake City, either dies or produces more flowering branches. Evidently induction of flowering in this annual is stronger than the reverse process, even in midsummer. The reverse process becomes evident in beet-seed fields of the Southwestern States when, owing to the fact that the temperatures have been too warm for adequate induction, some plants with seedstalks several feet in height turn vegetative. The same type of reaction is often evident with bolters in fields grown for sugar. It is on this account that the bolters classed as late are much less objectionable than those described as early (*6*). The flowering process in such plants is reversed, and nearly normal vegetative development of the roots takes place.

A knowledge of the principles of photothermal induction is useful to the plant breeder in breeding for a desired bolting tendency. Irregular germination under temperatures conducive to vernalization causes variability in degree of induction obtained. This may constitute a stumbling block to the breeder who selects for nonbolting without knowing that some of his nonbolting phenotypes failed to bolt merely because they were delayed slightly during the early period of germination. Nonbolting selections from early spring plantings may, on this account, be untrustworthy. Breeding for nonbolting is also handicapped by difficulties in obtaining photothermal induction adequate for propagation of the most extreme nonbolting selections. The strongest nonbolters are usually the poorest seed producers and tend to be neglected or dropped in the breeding process. Optimum and uniform conditions for induction of flowering are therefore required for the best results in developing nonbolting types.

A knowledge of the factors that influence photothermal induction is also of practical importance in the selection and maintenance of beet-seed-growing areas, particularly where the method (*21*) of overwintering the plants in the field is to be used. Success or failure in producing seed of some particular variety may depend on the choice of a suitable area. In some of the areas now growing seed, where temperatures are too high for the best thermal induction, it is important to develop promptly in the fall and hold an extensive growth of leaves to shade the soil and thereby reduce soil temperatures. The relation of the leaf area to induction by light may also be important, but this phase of the problem has not yet been adequately investigated.

SUMMARY

"Photothermal induction" of flowering is a new term used to signify induction of flowering by both light and temperature. In beets the effect of photoperiod was found to be intimately associated with and dependent upon temperature exposure. The effect of low-temperature exposure favorable to subsequent flowering was demonstrated

with germinating seed, with beets kept for a time in cold storage, and with growing plants.

Some of the factors that influenced bolting in the field acted indirectly by altering the range or duration of effective temperatures. Thus, shade increased bolting by lowering the temperature of the soil and consequently the temperature of the beet crown under conditions where the unshaded soil was too warm. Irregularity in germination of seeds under temperatures conducive to induction of flowering resulted in variation in bolting because of the fact that seeds retarded in sprouting escaped some of the low-temperature influence.

Genetic variability in beets with regard to response to temperature and photoperiod was shown in both annuals and biennials. A factor for bolting was identified, which is designated *B′* and is regarded as allelic to the factor *B* discovered by Munerati (*20*) and further described by Abegg (*1*). Identification of the factor *B′* was accomplished by hybridizing selected parental material and testing the backcross progenies under controlled environmental conditions.

LITERATURE CITED

(1) Abegg, F. A.
1936. A genetic factor for the annual habit in beets and linkage relationship. Jour. Agr. Res. 53: 493–511, illus.

(2) Čajlachjan, M. Ch.
1937. Concerning the hormonal nature of plant development processes. Acad. des Sci. U. R. S. S. Compt. Rend. (Dok.) (n. s.) 16: 227–230, illus.

(3) Carsner, Eubanks.
1928. The wild beet in California . . . Facts About Sugar 23: 1120–1121, illus.

(4) ———
1933. Curly-top resistance in sugar beets and tests of the resistant variety U. S. No. 1. U. S. Dept. Agr. Tech. Bul. 360, 68 pp., illus.

(5) Chroboczek, Emil.
1934. A study of some ecological factors influencing seed-stalk development in beets (Beta vulgaris L.). N. Y. (Cornell) Agr. Expt. Sta. Mem. 154, 84 pp., illus.

(6) Esau, Katherine.
1934. Bolting in sugar beets. A determination of its effect upon the weight and quality of the roots, based on studies in central California. Facts About Sugar 29: 155–158, illus.

(7) Fetter, Eugène.
1938. Quelques notes sur la jarovisation des graines de betteraves. Ann. de Gembloux 44: 319–326.

(8) Fisher, R. A.
1932. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.

(9) Garner, W. W., and Allard, H. A.
1920. Effects of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. 18: 553–606, illus.

(10) Hartsema, Annie, Luyten, Ida, and Blaauw, A. H.
1930. De optimale temperaturen van bloemaanleg tot bloei. (The optimal temperatures from flower formation to flowering.) K. Akad. van Wetensch. te Amsterdam, Verhandel. 27: 1–46, illus. [English summary, pp. 37–46.]

(11) Immer, F. R.
1930. Formulae and tables for calculating linkage intensities. Genetics 15: [81]–98, illus.

(12) Imperial Bureau of Plant Genetics.
1935. Vernalization and phasic development of plants. Imp. Bur. Plant Genet. Bul. 17, 151 pp.

(13) KELLER, WESLEY.
1936. INHERITANCE OF SOME MAJOR COLOR TYPES IN BEETS. Jour. Agr. Res. 52: 27–38.
(14) KNOTT, J. E.
1939. EFFECT OF TEMPERATURE ON THE PHOTOPERIODIC RESPONSE OF SPINACH. N. Y. (Cornell) Agr. Expt. Sta. Mem. 218, 38 pp., illus.
(15) LOEHWING, W. F.
1938. LOCUS AND PHYSIOLOGY OF PHOTOPERIODIC PERCEPTION IN PLANTS. Soc. Expt. Biol. and Med. Proc. 37: 631–634.
(16) ———
1938. PHYSIOLOGICAL ASPECTS OF SEX IN ANGIOSPERMS. Bot. Rev. 4: 581–625.
(17) MCKINNEY, H. H., and SANDO, W. J.
1935. EARLINESS OF SEXUAL REPRODUCTION IN WHEAT AS INFLUENCED BY TEMPERATURE AND LIGHT IN RELATION TO GROWTH PHASES. Jour. Agr. Res. 51: 621–641, illus.
(18) MELCHERS, G.
1937. DIE WIRKUNG VON GENE TIEFEN TEMPERATUREN UND BLÜHENDEN PFROPFPARTNERN AUF DIE BLÜHREIFE VON HYOSCYAMUS NIGER L. Biol. Zentbl. 57: 568–614, illus.
(19) MOŠKOV, B. S.
1937. PHOTOPERIODISM AND A HYPOTHESIS AS TO HORMONES OF FLOWERING. Acad. des Sci. U. R. S. S. Compt. Rend. (Dok.) (n. s.) 15: 211–214.
(20) MUNERATI, O.
1931. L'EREDITÀ DELLA TENDENZA ALLA ANNUALITÀ NELLA COMUNE BARBABIETOLA COLTIVATA. Ztschr. f. Zücht., Reihe A. Pflanzenzüchtung 17: [84]–89, illus.
(21) OVERPECK, JOHN C.
1928. SEED PRODUCTION FROM SUGAR BEETS OVERWINTERED IN THE FIELD. U. S. Dept. Agr. Cir. 20, 8 pp., illus.
(22) OWEN, FORREST V.; ABEGG, FRED A.; MURPHY, ALBERT M.; TOLMAN, BION; PRICE, CHARLES; LARMER, FINLEY G.; and CARSNER, EUBANKS.
1939. CURLY-TOP-RESISTANT SUGAR-BEET VARIETIES IN 1938. U. S. Dept. Agr. Cir. 513, 10 pp., illus.
(23) ROBERTS, R. H., and STRUCKMEYER, B. ESTER.
1938. THE EFFECTS OF TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS UPON THE PHOTOPERIODIC RESPONSES OF SOME OF THE HIGHER PLANTS. Jour. Agr. Res. 56: 633–677, illus.
(24) STEINBERG, ROBERT A., and GARNER, W. W.
1936. RESPONSE OF CERTAIN PLANTS TO LENGTH OF DAY AND TEMPERATURE UNDER CONTROLLED CONDITIONS. Jour. Agr. Res. 52: 943–960, illus.
(25) TCHERNETZKY, A. M.
1936. [EXPERIMENTS WITH VERNALIZATION OF SUGAR BEET SEED.] Selek. i Semen. 8: 51–56. [In Russian.]
(26) THOMPSON, H. C.
1929. PREMATURE SEEDING OF CELERY. N. Y. (Cornell) Agr. Expt. Sta. Bul. 480, 50 pp., illus.
(27) VOSS, J.
1936. EXPERIMENTELLE AUSLÖSUNG DES SCHOSSENS UND PRÜFUNG DER SCHOSSNEIGUNG DER RÜBENSORTEN (BETA VULGARIS L.). Angew. Bot. 18: 370–407, illus.
(28) WOODWARD, C. M.
1932. GENETICS AND BREEDING IN THE IMPROVEMENT OF THE SOYBEAN. Ill. Agr. Expt. Sta. Bul. 384, pp. 297–404, illus.

EFFECT OF BLUE STAIN ON SPECIFIC GRAVITY AND STRENGTH OF SOUTHERN PINE[1]

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INTRODUCTION

Blue stain, because of its widespread and common occurrence in the sapwood of logs and lumber, has been the subject of numerous investigations to determine its effect on the strength of wood. Among the earlier studies, between the years 1897 and 1911, were those by Rudeloff (*10*),[3] Von Schrenk (*12*), Münch (*9*), and Weiss and Barnum (*14*). The entire group included tests of compression parallel to the grain, bending strength, toughness, and hardness. The results were variable and on the whole rather inconclusive, probably owing in considerable measure to differences in the original mechanical properties of the wood and in the moisture content of the specimens at the time of testing. None of the results obtained in the several tests suggested that any practically significant weakening resulted from blue stain.

During the present decade the effect of blue stain on the strength of wood has received further attention, in most cases with more precise procedures to reduce the influence of factors other than the blue stain. In tests by Mayer-Wegelin and coworkers (*8*), Vanin (*13*), Chapman (*2*), Findlay and Pettifor (*3*, *4*), and Jalava (*7*), due consideration was given to the moisture content of the wood and, with the exception of that by Mayer-Wegelin et al., all or a substantial part of the work was done on matched [4] specimens in which stain had been produced by artificial inoculation and laboratory-controlled incubation. The results of these tests corroborated in general the findings of the earlier ones but disclosed definitely that blue stain may reduce the toughness of wood considerably. Except in one study by Findlay and Pettifor (*4*), on a tropical wood (obeche), compression and bending strength, hardness, and specific gravity have been reported as but slightly or not measurably affected.

The tests reported in this paper were conducted by the senior author and analyzed by the junior author. They conclude a series, the first results of which were published in 1933 (*2*). The aim of the additional testing was to gather evidence on additional fungi.

MATERIAL AND METHODS

SELECTION AND CUTTING OUT OF SPECIMENS

The test specimens were obtained from green logs of southern pine, taken at a height of 8 to 16 feet in the trees. The wood chosen was

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[3] Italic numbers in parentheses refer to Literature Cited, p. 132.

[4] The specimens were matched individually or by groups, by sawing them out of the same piece of wood in such a manner as would minimize the influence of the initial variation in physical properties.

straight-grained, clear throughout, and of medium density and uniform growth. The specimens were sawed to a size of ¾ by ¾ by 10 inches in such a manner as to present as nearly as possible true tangential and radial faces. Each specimen to be inoculated with a staining fungus was directly matched with a control specimen, either tangentially or longitudinally. The pattern of matching varied somewhat among the several tests, depending chiefly on the number of strength properties investigated at one time. However, there was no important deviation from the basic matching schemes, the essential features of which are illustrated in previous papers by the authors (*2*, *11*).

HANDLING AND STAINING OF SPECIMENS

All but certain specimens, which will be noted later, were maintained in the green condition, usually well above the fiber-saturation point, until the tests were concluded. Some of the tests were made on sticks that had been given no sterilization treatment prior to inoculation but, in order to insure pure cultures, most of the sticks were given a steam-heat treatment of 30 minutes at 100° C. and a few were heated for 5 minutes at a steam pressure of 12 pounds. Both the sticks that were subsequently stained and the controls were given the same heat treatment. In the absence of heat treatment, aseptic preparatory procedures and extra heavy inoculation were depended upon to reduce contamination. Visible contaminations were comparatively few and when they did occur the affected sticks were discarded.

The sticks to be stained were placed in large-bore test tubes and inoculated with heavy spore suspensions of the respective fungi. The cultures thus prepared were incubated at laboratory temperatures for 30 days, at the end of which time almost all the specimens were stained throughout and ready for testing. The uninfected controls were tested promptly after sawing, or, in the case of tests involving heat treatment of the wood, they were placed in preliminary storage for 30 days in sterile, closed containers. A fuller description of the handling and staining procedures appears in the first report (*2*).

STAIN FUNGI USED

The fungi used and the species of wood from which they were isolated are listed in table 1. *Ceratostomella pilifera* (Fr.) Wint. and *Graphium rigidum* (Pers.) Sacc. are among the predominant species staining the sapwood of pine and red gum lumber, respectively.

TABLE 1.—*Test fungi and species of wood from which they were isolated*

Fungus	Culture No.	Original wood substrate
Ceratostomella pilifera	3	Shortleaf pine (*Pinus echinata* Mill.).
C. pilifera	185	Longleaf pine (*P. palustris* Mill.).
C. pilifera	186	Loblolly pine (*P. taeda* L.).
C. pini	202	Shortleaf pine.
C. pini	212	Ponderosa pine (*P. ponderosa* Dougl.).
C. ips	157	Longleaf pine.
C. ips	202	Shortleaf pine.
Graphium rigidum	198	Red gum (*Liquidambar styraciflua* L.).

C. pini Münch and *C. ips* Rumbold are primarily associated with stain accompanying certain beetle infestations of pine trees, but *C. ips* is also a common independent cause of stain in southern pine logs and lumber.

TESTING PROCEDURE

Tests were made of compression parallel to the grain, toughness, and, in some cases, of static bending. In addition, the specific gravity of each specimen was determined from its dimensions, weight, and moisture content when tested. Conventional machines were employed for the compression and bending tests and a special impact machine of the pendulum type, developed at the Forest Products Laboratory, was used for tests of toughness. In the bending and toughness tests loads were applied at the center of an 8-inch span. The compression tests were made on pieces 2½ inches long taken from the ends of the specimens used in the toughness tests but well removed from the zone of fracture.

ANALYSIS OF DATA

The effect of staining on the several wood properties is appraised on the basis of the geometric mean ratios of stained wood to control values. By virtue of the matching of stained specimens and controls in pairs, it is assumed that any differences between the members of a pair due to factors other than the blue stain were essentially equalized. A ratio is held to be significantly different from 1 if its logarithm is significantly different from 0.[5] (A mean value of 1, of course, would indicate no difference between the stained wood and the controls.)

PROPERTIES OF WOOD AS AFFECTED BY BLUE STAIN

TOUGHNESS AND WORK TO MAXIMUM LOAD

The results of the tests are summarized in table 2. Values indicating a reduction in a particular property that individually are mathematically significant on the basis stipulated above are marked by asterisks. Considering first the group of tests as a whole, the results are in good agreement with previous reports of similarly controlled experiments. Toughness and work to maximum load are both regarded as an index of shock resistance and were the only properties analyzed that were greatly affected. The greatest significant reduction in toughness amounted to about 75 percent and the smallest to about 9 percent. Similarly, the greatest significant reduction in work to maximum load amounted to about 41 percent and the smallest to about 14 percent. Had total work been included it presumably would have corresponded more closely to the toughness values, as was the case in earlier tests (*2*).

[5] Because there is no theoretical or known reason why any effect of blue stain fungi on the wood properties studied should be other than in the direction of a reduction, and any differences due to staining, therefore, can be regarded as fixed with respect to sign, a 10-percent level of probability is taken as the basis for appraising significance rather than its equivalent—a probability of 5 percent without regard to the direction of the difference. The authority referred to for this procedure is Goulden (*6*).

TABLE 2.—*Specific gravity and strength of blue-stained pine sapwood*

Test No.	Stain fungus	Culture No.	Wood species	Number of matched pairs of test specimens	Average relative value for stained wood [1] (value for matched controls=100)				
					Specific gravity [2]	Compression parallel to grain	Static bending		Toughness
							Modulus of rupture	Work to maximum load	
TEST SPECIMENS NOT GIVEN STERILIZATION TREATMENT									
5	*Ceratostomella pilifera.*	185	*Pinus palustris.*	12	98.8	104.4	100.0	*71.0	*55.1
6	do	185	do	18	*97.9	*93.8	*96.7	*74.9	*59.2
2	*Graphium rigidum*	198	*P. taeda*	16 to 18	98.5	97.0			94.4
3	do	198	*P. echinata*	14 to 16	98.4	*89.8	99.0	94.2	*75.2
4	do	198	*P. taeda*	24 to 25	100.8	100.9	*95.6	*75.7	*76.0
TEST SPECIMENS STEAMED 30 MINUTES AT 100° C.									
1	*C. ips*	157	*P. taeda*	19	100.0	102.4			*90.6
1	do	202	do	20	99.6	102.2			*52.9
1	*C. pilifera*	3	do	18	98.9	105.2			*63.1
1	do	186	do	20	99.3	110.3			*58.8
1	do	185	do	21	98.9	101.1			*29.4
5	do	185	*P. palustris*	12	99.3	111.2	99.1	*58.6	*36.8
6	do	185	do	18	*97.4	98.7	102.3	*64.6	*43.2
1	*C. pini*	202	*P. taeda*	20	99.4	105.6			*70.0
1	do	212	do	18	*98.6	109.6			*70.2
1	*G. rigidum*	198	do	21	99.3	99.5			*26.3
2	do	198	do	16 to 18	*96.2	*93.1			*24.6
3	do	198	*P. echinata*	14 to 16	101.8	*95.1	*90.0	*75.0	*76.5
4	do	198	*P. taeda*	24 to 25	*97.0	99.6	*95.6	*71.7	*57.3
TEST SPECIMENS STEAMED 5 MINUTES AT 12 POUNDS' PRESSURE									
2	*G. rigidum*	198	*P. taeda*	16 to 18	*96.9	*90.8			*24.4
3	do	198	*P. echinata*	14 to 16	100.0	101.8	*97.4	*85.6	*83.4

[1] Derived from the geometric means of the ratios: $\frac{\text{Value for control specimen}}{\text{Value for stained specimen}}$. Values that individually are significantly less than 100 are designated by asterisks (*). Certain values are significantly greater than 100, but as a result of moisture differences rather than the blue stain.

[2] Based on green volume and oven-dry weight.

SPECIFIC GRAVITY

In only 6 of the tests was the stain accompanied by a significant reduction in specific gravity. Nevertheless, in 16 tests out of the total of 20, the specific gravity of the stained wood was no less than 0.4 percent lower than that of the controls and in most of these 16 cases it was at least 1 percent lower. Mathematical analysis of the aggregate result [6] indicates that there was a definite tendency toward specific gravity reduction by the several stain fungi. In the 6 tests where specific gravity losses were individually significant, the greatest reduction was 3.8 percent and the smallest 1.4 percent.

COMPRESSION

The effect of staining on compression parallel to the grain was rather variable; in many cases an increase in this property was noted. However, significant increases occurred only in tests 1 and 5, in which a number of the stained compression specimens were found at the time of

[6] According to Fisher's (*5, p. 104*), treatment of the combination of probabilities from tests of significance (suggested to the writers by R. A. Chapman).

testing to be drier than those in the other tests, the moisture content being substantially less than in the controls and frequently near the fiber-saturation point. It is therefore assumed that moisture differences, rather than any effect of staining, were responsible for the significant increases in compression parallel to the grain.[7] The five significant reductions in compression strength all fell within a range of about 5 to 10 percent; hence were comparatively substantial. It should be noted, however, that these results were, with one exception, by the same fungus, and therefore are not necessarily representative of the group.

MODULUS OF RUPTURE

Individually significant reductions in modulus of rupture, ranging from about 3 to 10 percent, occurred in five of the nine tests of this property, and lesser, not individually significant, reductions occurred in two of the tests. As in the case of specific gravity, the aggregate result was a statistically significant reduction in this property.

SPECIFIC ACTION OF FUNGI

The four species of blue stain fungi did not affect the wood with equal severity, and the same fungus did not have the greatest effect on all properties. *Ceratostomella pilifera* reduced the toughness of the unheated wood and the work to maximum load of the heated wood appreciably more than did *Graphium rigidum*. On the other hand, *G. rigidum* had the greater effect in the case of modulus of rupture of the heated wood. Definitely assignable differences between the effects of these two fungi were not brought out by the other tests. *C. ips* and *C. pini*, although they produced a more intense stain than *C. pilifera* or *G. rigidum*, on the whole did not weaken the wood as much.

In line with some of the results previously reported, staining tended to have a greater effect on the heated wood than on the unheated. Differences in this respect were not evidenced in all properties, but mainly by work to maximum load and toughness. The tests were not replicated sufficiently to permit a quantitative analysis of the effects of heating.

DISCUSSION AND CONCLUSIONS

Reductions in compression and bending strength as great as some of those reported by Chapman (*2*) and again encountered in these tests appeared to be sufficiently unusual to warrant a special search for their cause. The answer apparently lies to a considerable extent in the respective abilities of the several fungi to extend their development beyond the wood rays, upon which their attack typically is centered, and to penetrate directly the walls of the tracheids. Microscopic examination disclosed that direct cell-wall penetration was common with *Graphium rigidum*, somewhat less so with all strains of *Ceratostomella pilifera*, and only occasional with the strains of *C. ips* and *C.*

[7] Losses of moisture from the test specimens took place largely during the incubation periods and, of course, were greatest from portions nearest the mouth of the culture tubes. It was for this reason that the stained compression specimens, which were taken near one end of the toughness specimens, tended to have a lower moisture content; the controls, having been tested immediately or else stored in closed containers, did not have the same opportunity to dry out as the stained pieces. Except for some of the compression specimens of tests 1 and 5, the moisture content of the pieces remained high enough not to interfere with the test results. An increase in the strength of wood as it dries does not occur until the moisture content falls approximately to the fiber-saturation point; practically speaking, the point where the cell walls are saturated but no free water is present in the cell cavities.

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pini. An abundance of *G. rigidum* hyphae in the tracheids regularly penetrating the walls also offers a tenable explanation for reductions in specific gravity by this fungus amounting in one case to almost 4 percent. Findlay and Pettifor (*3*) have called attention to the fact that the reduction in specific gravity resulting strictly from a destruction of food materials stored in the wood rays probably would not exceed about 2 percent in Scots pine.

The microscopic examination further brought out what appeared to be a definite relation between the relative abundance of cell-wall perforations by a particular fungus and the toughness of the wood. This relationship extended in part to the different species of fungi but did not hold for *Ceratostomella pilifera* and *Graphium rigidum*. It is assumed, of course, that a large proportion of the reduction in toughness in all cases was accomplished by the typical operations of the fungi strictly within the ray cells, as was essentially the case with *C. ips* and *C. pini*. Further evidence for the correctness of this assumption is offered by the fact that certain decay fungi may markedly affect the toughness of wood without penetrating the walls of the wood fibers or tracheids (*1, 11*).

In view of the results of the present study, it is rather surprising that *Graphium rigidum* plays a somewhat minor part in the staining of pine lumber whereas *Ceratostomella ips* is one of the predominant blue stain species. This leads to the generalization that predominance of a particular stain fungus in a certain kind of wood may not necessarily signify a greater local effect on the wood properties than that of a less prominent fungus. However, it is known that different strains of fungi may differ variously in their effect on wood. Hence the results with a comparatively few strains should not be interpreted too closely.

While the intensity of discoloration may be a criterion of the intensity of attack and amount of strength reduction caused by a single species of blue stain fungus, it apparently is not necessarily indicative for different species. As already mentioned, *Ceratostomella ips* and *C. pini* produced the darkest stain but otherwise affected the wood least. On the other hand, the extent of staining (i. e., amount of discolored wood) is a fairly reliable index of the magnitude of fungus attack and therefore might have some value as a basis for estimating relative strength losses, irrespective of the fungus or fungi concerned. For example, results obtained by Findlay and Pettifor, with naturally infected pinewood, indicated that loss in toughness was roughly proportional to the extent of staining. Specimens having 0 to 39 percent of the cross-sectional areas stained retained about 94 percent of their original toughness, whereas specimens with 70 to 100 percent of the cross-sectional area stained retained about 88 percent of their original toughness. However, with a little more than one-third of the cross-sectional area stained, about one-half of the maximum toughness reduction occurred. This result, if representative, possibly would place excessive demands on the accuracy of estimation.

In considering the practical aspects of blue stain, it should be kept in mind that the preceding results and those of the more recent previous investigations are based to a considerable extent on wood that had received some degree of heat sterilization. The factor of heating, as well as others introduced by laboratory experimentation, raises the question of how nearly results so obtained are representative of the

blue-stained wood ordinarily encountered. Greater strength losses in heated wood have been explained by assuming that the heat produced changes in the wood, rendering more of it immediately available to the fungi. However, with sufficient time and favorable conditions, the fungi themselves may effect some of the nutritionally significant changes created by mild heating, in which case the ultimate effects of staining in slowly air-dried wood might approach those found in the heated wood. The critical period for stain occurrence in air-dried lumber is generally believed not to exceed about 30 days in the majority of cases. Consequently, in air-seasoned board stock at least, strength reductions caused by blue stain are probably variously less than the tests on the steamed wood indicate. Chapman (*2*) reported that stained lumber naturally infected on the seasoning yard revealed less direct cell-wall penetration than existed in heated test specimens. Furthermore, he found that values for the wood stained by *Graphium rigidum* and *Ceratostomella pilifera*, in tests that incorporated a heat treatment consisting in all but one case of 30 minutes at 100° C., were lower than those in tests with no heat treatment by approximately the following percentages: Specific gravity, 0; modulus of rupture, 4; total work in bending, 30; toughness, 29.

Sufficient data have been accumulated to indicate rather definitely that blue stain, at least in unheated pinewood, reduces most strength properties comparatively little and need not be a cause for discrimination from this standpoint except where strength, particularly shock resistance, is a prime requisite. Considering, necessarily somewhat subjectively, the results of the present and prior experiments, including those by others, the authors believe that fully developed blue stain in naturally infected pinewood commonly may reduce specific gravity 1 to 2 percent, strength in compression parallel to the grain and modulus of rupture 1 to 4 or 5 percent, and toughness 15 to 30 percent. Surface hardness may be reduced 2 to 10 percent (*3*, *7*). The duration of favorable conditions for staining and the particular fungus or fungi involved apparently are the most important factors determining the extent of weakening.

Exceptions to these conclusions may be found in certain woods other than pine, as for example, in obeche wood studied by Findlay and Pettifor (*4*). Obeche specimens stained by *Botryodivlodia theobromae* lost as much as 43 percent of toughness, 19 percent of bending strength, and 12 percent of specific gravity.

It may be significant that, with a reduction in specific gravity of about 2 percent, the corresponding reduction in strength caused by blue stain fungi does not appear to be a great deal less than that caused by a vigorously attacking decay fungus; in fact, there is substantial evidence that the toughness reduction may be even greater in some cases. For example, in laboratory decay tests by one of the authors (*11*), conducted in practically the same manner as the present stain study, the average percentage losses in strength associated with a 2-percent reduction in the specific gravity of red gum infected with *Polyporus versicolor* were about as follows: Compression parallel to grain, 7; modulus of rupture, 10; toughness (total work), 35–40; and hardness, 7.

To complete the discussion of the practical aspects of blue stain it should be pointed out that, in general, the conditions that favor the development of stain fungi also favor the development of decay

fungi; hence it is often desirable to examine heavily stained material for signs of decay. Important decay infection does not necessarily accompany heavy and extensive blue stain, since blue stain typically precedes decay, but that it commonly may do so is suggested by a recent report (*15*) in which the strength of heavily stained lumber was compared with that of comparably seasoned lumber that had been kept bright by chemical treatment. Reductions in the strength of the stained lumber in some cases were greater than could be accounted for by the presence of the stain alone.

SUMMARY

The effect of blue stain on the specific gravity and strength of pine wood artificially inoculated with pure cultures of four species of fungi and with different strains of some of them, was essentially the same as reported for prior, similarly controlled studies. Although all strength properties appeared to be lowered generally, only toughness was affected to an extent of general practical significance.

The different species of blue stain fungi did not affect the wood with equal severity, nor was their relative order of superiority in this respect entirely the same for all properties tested. There was some evidence of a broad relation between the abundance of direct cell-wall penetration by the fungi and reduction in toughness. The intensity of the discoloration caused by the different fungi was not indicative of the severity of attack nor of the comparative weakening caused by each. The frequency of association between a particular blue stain fungus and a certain kind of wood apparently does not necessarily indicate the inherent ability of the fungus to attack the wood and to affect its strength.

LITERATURE CITED

(1) Cartwright, K. St. G., Campbell, W. G., and Armstrong, F. H.
1936. The influence of fungal decay on the properties of timber. I. The effect of progressive decay by polyporus hispidus, fr., on the strength of english ash (fraxinus excelsior, l.). Roy. Soc., London, Proc., Ser. B. 120: 76–95, illus.

(2) Chapman, A. Dale.
1933. Effect of steam sterilization on susceptibility of wood to blue-staining and wood-destroying fungi. Jour. Agr. Res. 47: 369–374, illus.

(3) Findlay, W. P. K., and Pettifor, C. B.
1937. The effect of sap-stain on the properties of timber. I. Effect of sap-stain on the strength of scots pine sapwood. Forestry 11: 40–52.

(4) ——— and Pettifor, C. B.
1939. Effect of blue stain on the strength of obeche (triplochiton scleroxylon). Empire Forestry Jour. 18 (2): 259–267, illus.

(5) Fisher, R. A.
1936. Statistical methods for research workers. Ed. 6, rev. and enl., 339 pp., illus. Edinburgh and London.

(6) Goulden, C. H.
1939. Methods of statistical analysis. Rev. ed., 277 pp., illus. New York and London.

(7) Jalava, Matti.
1938. Effect of blue stain on the strength of pine timber. Helsinki, Teknillisiä Tiedonantoja (Finland Found. for Forest Prod. Res.), No. 8, 24 pp., illus. [In Finnish. English summary, pp. 22–24. Also abstract in Chem. Abs. 33: 6018. 1939.]

(8) MAYER-WEGELIN, H., BRUNN, G., and LOOS, W.
1931. ZUR FRAGE DER BEWERTUNG STAMMBLAUEN KIEFERNHOLZES. Mitt. aus Forstw. u. Forstwiss. pp. 573–588, illus.
(9) MÜNCH, ERNST.
1907–8. DIE BLAUFÄULE DES NADELHOLZES. Naturw. Ztschr. f. Forst- u. Landw. 5: 531–573; 6: 32–47, 297–323, illus.
(10) RUDELOFF, M.
1897, 1899. UNTERSUCHUNGEN ÜBER DEN EINFLUSS DES BLAUWERDENS AUF DIE FESTIGKEIT VON KIEFERNHOLZ. Teil 1–2. Mitt. aus den K. Tech. Versuchsanst. 15: 1–46, 1897; 16: 209–239, illus., 1899.
(11) SCHEFFER, THEODORE C.
1936. PROGRESSIVE EFFECTS OF POLYPORUS VERSICOLOR ON THE PHYSICAL AND CHEMICAL PROPERTIES OF RED GUM SAPWOOD. U. S. Dept. Agr. Tech. Bul. 527, 46 pp., illus.
(12) SCHRENK, HERMANN VON.
1903. THE "BLUING" AND THE "RED ROT" OF THE WESTERN YELLOW PINE, WITH SPECIAL REFERENCE TO THE BLACK HILLS FOREST RESERVE. U. S. Bur. Plant Indus. Bul. 36, 40 pp., illus.
(13) VANIN, S. I.
1932. BLUE STAIN OF TIMBER AND HOW TO CONTROL IT. 103 pp., illus. Moscow, Leningrad. [In Russian.]
(14) WEISS, HOWARD F., and BARNUM, CHARLES T.
1911. THE PREVENTION OF SAP STAIN IN LUMBER. U. S. Forest Serv. Cir. 192, 19 pp., illus.
(15) WILSON, T. R. C., and RICHARDS, C. AUDREY.
1938. DIPPING HELPS TO CONSERVE STRENGTH OF LUMBER. Amer. Lumberman 3126: 68; Hardwood Rec. 76 (5): 30; South. Lumberman 156 (1937): 30; Timberman 39 (7): 44.

THE IMPAIRMENT IN NUTRITIVE VALUE OF CORN GRAIN DAMAGED BY SPECIFIC FUNGI [1]

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INTRODUCTION

It has been estimated (*6*) [2] that the corn crop of the State of Illinois suffers an average annual loss of at least 20 percent from diseases of all kinds. The total loss from ear rot is assessed at 7.5 percent, equivalent in terms of annual production to 23 million bushels. In this estimate rot-damaged kernels were considered as a loss.

The extent of infection of the corn grain by fungi of various kinds is an important item in the grading of corn either by physical or chemical means (*12*) or by performance tests (*3*). However, in view of the fact that the preponderant proportion of the corn crop in this country is used as animal feed, the extent to which a crop should be penalized for its content of unsound corn should depend upon the impairment in nutrituve value that has resulted from the type of damage incurred.

Moldy corn may be toxic to pigs (*5*) and horses (*9*), and, possibly, in large quantities, to chickens (*8*), but the investigations referred to cannot be taken to mean that all moldy corn is toxic, or that any particular mold will produce a toxic grain, because unfortunately the organism responsible for the toxicity, whether fungus or bacterium, was not identified. In other experiments (*1, 11*), molds of the genus *Fusarium* have been definitely and causally related to the toxicity of infected corn.

Insofar as the writers are aware, no experiments on the nutritive value of unsound as compared with sound corn have been reported in the literature. Hence, at the suggestion of Dr. George H. Dungan of the Agronomy Department of the University of Illinois, the experiments described herein were undertaken, having as their object the determination of the effect on the protein and energy values of the corn kernel of infection with specific fungi. The fungi chosen for study were *Diplodia zeae* (Schw.) Lev., *Fusarium moniliforme* Sheldon, and *Gibberella zeae* (Schw.) Petch [*G. saubinetii* (Mont.) Sacc.]. These fungi are arranged in the order of the seriousness of the damage they produce on the corn crop in Illinois (*6*), although at times and in certain sections of the State, fusarium ear rot has accounted for significant damage to more than 25 percent of the ears.

EXPERIMENTAL MATERIALS [3] AND METHODS

The ears of corn (*Zea mays* L.) were inoculated by hand with pure spores of the organisms. When harvested, the kernels shelled from the ears infected with the diplodia and gibberella organisms were

[1] Received for publication December 22, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 140.

[3] The material used in these experiments was provided through the generous cooperation of Dr. Benjamin Koehler of the Department of Agronomy.

damaged to the extent of 94 to 98 percent in the former case, but only 53 percent in the latter. In the case of the fusarium-damaged corn, because of the spotted nature of the infection, the damaged kernels were separated by hand. For each sample of damaged corn, a sample of sound corn exhibiting a very inappreciable damage from mold was secured for comparative purposes from the same field and crop. All samples were analyzed chemically and their heat of combustion determined in the bomb calorimeter. The results are compiled in table 1.

TABLE 1.—*Varieties, chemical composition, and gross energy value of sound and fungus-damaged corn samples tested*

Variety [1]	Condition	Weight of 100 kernels	Moisture	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Gross energy per gram	Ash	Phosphorus	Calcium
		Grams	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Small calo-ries*	*Per-cent*	*Per-cent*	*Per-cent*
Illinois hybrid 947	Sound	23.828	9.72	11.12	4.10	1.94	71.82	4,093	1.40	0.328	------
	Diplodia rot	20.046	10.06	11.69	2.64	2.51	71.58	4,039	1.52	.350	------
Illinois hybrid 92	Sound	25.085	9.54	10.88	4.00	1.76	72.43	4,082	1.39	.323	0.0050
	Diplodia rot	21.370	9.58	11.19	3.08	2.52	72.10	4,040	1.53	.330	.0061
Illinois hybrid 172	Sound	23.605	9.78	10.75	4.26	1.74	72.10	4,084	1.37	.311	------
	Diplodia rot	21.569	10.46	11.00	3.12	2.31	71.48	4,003	1.63	------	------
Iowa hybrid 13	Sound	31.139	9.81	10.94	3.74	1.78	72.29	4,047	1.44	------	------
	Diplodia rot	23.709	10.22	11.69	2.60	2.26	71.67	4,005	1.56	------	------
Illinois hybrid 960	Sound	26.294	8.96	7.69	4.46	1.92	75.69	4,019	1.28	.276	.0033
	Diplodia rot	21.522	8.83	7.62	2.82	2.46	76.89	3,962	1.38	.308	.0060
Improved Reid Yellow Dent.	Sound	32.590	8.84	8.88	4.65	1.91	74.37	4,060	1.35	.312	.0023
	Diplodia rot	25.874	8.90	9.12	3.06	2.50	74.97	3,994	1.45	.318	.0043
Do	Sound	23.546	9.72	10.62	4.30	1.72	72.34	4,077	1.30	.303	.0060
	Diplodia rot	22.664	9.60	12.19	2.90	2.64	71.06	4,055	1.61	.366	.0072
	Gibberella rot	17.414	9.56	13.00	3.94	2.94	68.71	4,113	1.85	.385	.0078
Do	Sound	30.268	7.46	9.00	4.31	1.98	75.81	4,127	1.44	.228	.0038
	Fusarium rot	20.835	8.46	9.88	3.57	2.60	73.87	4,064	1.62	.258	.0087

[1] The hybrids had the following pedigrees: Illinois hybrid 947, (R4 × Pr) × Tr × 317); Illinois hybrid 92, (WF9 × 5120)×(R4 × Hy); Illinois hybrid, 172, (R4 × Hy)×(A × 540); Illinois hybrid 960, (R4 × Hy)×(317 × 701); Iowa hybrid 13, (349 × 317)×(345 × 401). The improved Reid Yellow Dent was the Illinois Agricultural Experiment Station strain of this variety.

For the protein tests, three rations were made up for each pair of corn samples tested. Two of the rations contained 88 and 87 percent of the sound and unsound corn, respectively, together with vitamin and mineral supplements. The lower oil content of the damaged corn was compensated for by the inclusion of 1 percent of corn oil in the corresponding ration so that the gross energy values would be approximately equal. These rations contained only from 9.5 to 12.5 percent of crude protein, mostly derived from the comparative corn samples, but were otherwise complete. The third, or diluting, ration contained no corn at all, but the same mineral and vitamin supplements as the other two, and enough starch, sucrose, and corn oil to furnish approximately the same gross energy per gram.

The rations for the energy tests contained 68 and 67 percent of sound and unsound corn, respectively, and were supplemented, not only with vitamins and minerals, but also with 20 percent of casein. They were, therefore, nutritively complete, closely similar in gross energy value by actual determination with the bomb calorimeter, and possibly different only in the extent of utilization of the contained energy. The composition of the rations is shown in table 2.

The paired-feeding technique was used throughout these experiments. In the energy tests, paired rats were fed equal amounts of food and the relative energy values were judged by the gains in body weight produced and by the gross energy content of the carcasses of pair mates. In the protein tests, considerable differences in live weight of paired rats were not allowed to develop. As soon as a tendency for a difference in weight appeared, the ration of the rat gaining the faster was diluted with the diluting ration above described, containing only about 0.5 percent of nitrogen, to the extent required to maintain equality in weight between pair mates. Differences in protein quality of comparable rations were, under these conditions, inversely proportional to the amounts of dietary protein required for equal gains in body weight.

The rats were started generally at weights varying between 40 and 55 gm. The digestibility of dietary nitrogen or of dietary energy was determined in each experiment in a 7-day collection period on constant intake of food, a marker (F_2O_3) being used to separate the feces. At the termination of the feeding periods, which lasted usually for 6 to 12 weeks depending upon the rates of growth secured, the rats were killed with ether, the body lengths from tip of nose to root of tail measured, and the empty body weights determined. In one experiment (No. 168) concerned with the energy value of sound and fusarium-infected corn, the carcasses of the experimental rats were frozen, ground finely in a hand mill, thoroughly mixed, and the gross energy (heat of combustion) of weighed portions determined in the bomb calorimeter.

TABLE 2.—*Composition of the experimental rations used in the protein and the energy studies with sound and fungus-damaged corn*

Ingredients	Protein studies			Energy studies	
	Sound corn ration	Unsound corn ration	Diluting ration	Sound corn ration	Unsound corn ration
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Sound corn	88.0	0.0	0	68.0	0
Unsound corn	0	87.0	0	0	67.0
Salt mixture	4.0	4.0	4.0	4.0	4.0
Dried yeast	6.0	6.0	6.0	6.0	6.0
Cod-liver oil	1.5	1.5	1.5	1.5	1.5
Wheat-germ oil	.5	.5	.5	.5	.5
Corn oil	0	1.0	4.0	0	1.0
Starch	0	0	74.0	0	0
Sucrose	0	0	10.0	0	0
Casein	0	0	0	20.0	20.0

EXPERIMENTAL RESULTS

The analyses of the samples of sound and unsound corn, collected in table 1, together with the weights of 100 kernels in each case, reveal some fairly consistent differences. In all comparable samples, the mold-damaged corn contained less fat, averaging about 25 percent less, than the sound corn, more ash, calcium, and phosphorus, distinctly more crude fiber, and generally more nitrogen. These differences must result from the destructive action of the mold on some of the nutrients in the corn and from the addition of the nutrients of the mold itself. The germ seems to be the most vulnerable part of the kernel for most

types of damage; of the constituents of the germ, it has been shown by Rabak (*7*) and Zeleny and Coleman (*12*) that the oil is definitely destroyed by molds. In fact, Zeleny and Coleman found that fat acidity is a satisfactory chemical measure of soundness in corn, being more reliable even than the percentage of damaged kernels. The lower content of fat (ether extract) in the damaged corn than in the sound corn can probably be explained, therefore, on the basis of a direct destruction of fat by the fungus. The increased percentage content of protein (N×6.25), crude fiber, and ash in the damaged corn may be in part a mathematical resultant of the lowered percentage of fat in the germ and in part a distortion of the proportions normally existing among the anatomical parts of the corn kernel. These increases are analogous to the increased content of carotenoids that Clark and Gring (*2*) observed in damaged corn, though in the latter case a possible differential effect of storage for more than a year on the sound and the damaged grain must also be considered.

TABLE 3.—*Average results of the protein studies when sound and fungus-damaged samples of different varieties of corn were fed to rats*

Experiment No.	Variety of corn	Condition of corn	Pairs of rats used	Average time on feed	Average total gain in weight	Average body length	Digestibility of nitrogen [1]		Ratio of nitrogen intake [1]	
							Average	Pairs conforming	Average	Pairs conforming
			Number	*Days*	*Grams*	*Millimeters*	*Percent*	*Number*		*Number*
124	Illinois hybrid 92	Sound	4	48.5	57.6	172.8	84.2	3	1	4
		Diplodia rot			56.4	171.8	82.5		1.22	
162	Improved Reid Yellow Dent.	Sound	6	38.8	40.5	169.2	83.5	5	1	6
		Diplodia rot			39.3	170.5	81.8		1.34	
139	Iowa hybrid 13	Sound	9	69.2	60.8	177.5	85.1	8(8)	1	8(8)
		Diplodia rot			61.7	177.2	81.9		1.18	
143	Illinois hybrid 947	Sound	10	65.9	62.5	178.3	84.1	7(8)	1	10
		Diplodia rot			61.6	177.0	80.6		1.27	
144	Illinois hybrid 172	Sound	8	71.9	71.1	179.6	84.2	7.5	1	7(7)
		Diplodia rot			69.6	179.7	82.6		1.25	
206	Improved Reid Yellow Dent.	Sound	10	70.6	85.1	185.9	82.8	8(9)	1	10
		Diplodia rot			84.4	185.4	79.6		1.25	
208	Illinois hybrid 960	Sound	10	71.2	67.4	175.2	81.6	8	1	8
		Diplodia rot			66.6	176.2	79.4		1.13	
119	Improved Reid Yellow Dent.	Sound	8	5.6	−12.4					
		Gibberella rot.			−15.1					
167	do	Sound	10	46.5	18.1	155.0	81.9	10	1	10
		Fusarium rot.			17.1	151.3	75.0		1.34	

[1] When the number of determinations of digestibility and of the ratio of nitrogen intakes required for equal gains is less than the number of pairs of rats originally placed on experiment (column 4), the reduced number is indicated in parentheses.

The average results of the protein studies are collected in table 3. The statistical significance of the differences between averages is indicated by the "number of pairs conforming," given in columns 9 and 11. Thus, in experiment 124, of the four pairs of rats used, a greater digestibility of nitrogen was observed in three pairs, and a smaller intake of nitrogen required in all four pairs by the rat on the sound corn ration. In this experiment, 1 gm. of nitrogen in the sound corn proved to be the nutritive equivalent of 1.22 gm. of nitrogen in the diplodia-rotted grain.

The evidence in table 3 demonstrates that the diplodia fungus definitely, if only slightly (about 2.5 percent), depressed the digestibility of protein in the corn kernel and lowered its biological value. On an average, 1 gm. of nitrogen in the sound corn was the equivalent of 1.23 gm. of nitrogen in the diplodia-damaged corn, a difference that cannot be accounted for, except in small part, by the impairment in digestibility observed. This difference indicates that the nitrogen in the damaged corn was only 81.3 percent $[(1 \div 1.23) \times 100]$ as valuable to the growing rat as the nitrogen in the sound corn.

The fusarium-damaged corn showed a greater impairment in the digestibility and in the total nutritive value of its nitrogen for maintenance and growth than the diplodia-damaged corn. The depression in digestibility averaged 6.9 percent; and in the promotion of growth, 1 gm. of nitrogen in the sound corn was the nutritive equivalent of 1.34 gm. of nitrogen in the damaged corn. This means that the nitrogen of the damaged corn was only 74.7 percent as valuable to the growing rat as the nitrogen in the sound corn. However, it cannot be inferred that fusarium rot is more deleterious to the nutritive value of corn than diplodia rot, because in the particular samples tested the extent of damage may not have been comparable.

The protein damage occasioned by the diplodia and fusarium fungi may be entirely the result of the conversion of the proteins of the corn kernel into mold proteins, which have been shown to be of very low biological value (*4, 10*). This explanation is offered on the assumption that all molds are similar in the nutritive value of their proteins for the support of mammalian growth, the references cited having to do with other molds than those here studied.

The gibberella-damaged corn proved to be so toxic for rats that too little was eaten to permit a fair test. However, even with this limited consumption of the ration containing the damaged corn, many of the rats died.

TABLE 4.—*Average results of the energy studies when sound and fungus-damaged samples of different varieties of corn were fed to rats*

Experiment No.	Variety of corn	Condition of corn	Pairs of rats used	Average time on feed	Gain in body weight		Body length		Digestibility of energy	
					Average	Pairs conforming [1]	Average	Pairs conforming [1]	Average	Pairs conforming [1]
			Number	*Days*	*Grams*	*Number*	*Millimeters*	*Number*	*Percent*	*Number*
157	Illinois hybrid 947	Sound	10	66.2	141.5	9	199.2	6.5	90.2	10
		Diplodia rot			133.6		200.2		88.9	
205	Improved Reid Yellow Dent	Sound	10	84	149.9	8.5	202.0	6.5	90.5	10
		Diplodia rot			143.1		201.9		89.2	
207	Illinois hybrid 960	Sound	10	77	156.7	9.5	204.5	5	91.4	9
		Diplodia rot			148.8		205.1		90.4	
168	Improved Reid Yellow Dent	Sound	10	56	57.2	10	175.8	10	91.0	10
		Fusarium rot			46.4		168.4		88.9	

[1] See text for further explanation

The average results of the energy studies on diplodia-damaged and fusarium-damaged corn are given in table 4. Again the evidence of nutritive damage is clear-cut. The gains in body weight and the

digestibility of energy were definitely depressed on the rations containing unsound corn as compared with the rations containing sound corn. Here, also, the damage associated with fusarium infection was definitely greater than that associated with diplodia infection.

To complete the evidence with reference to impairment of energy utilization, the rats in experiment 168 were killed and the energy content of their carcasses determined, with the results assembled in table 5. In all 10 pairs the rat on the unsound corn contained less gross energy than the rat on the sound corn, the average deficit amounting to 25 calories, or 13 percent.

TABLE 5.—*The energy content of the rats used in experiment 168*

Pair No.	Energy content of carcass in—			
	Calories per gram [1] of rats fed—		Total calories of rats fed—	
	Sound corn ration	Unsound corn ration	Sound corn ration	Unsound corn ration
	Calories	*Calories*	*Calories*	*Calories*
1	1.93	2.00	179	174
2	1.99	1.83	181	137
3	1.90	1.81	145	122
4	1.96	1.91	218	183
5	2.03	1.70	219	159
6	2.09	1.97	236	220
7	1.86	2.07	186	184
8	1.68	1.91	197	197
9	1.97	2.03	164	156
10	2.06	1.74	202	151
Average	1.95	1.90	193	168

[1] Calculated on empty weight of rats.

CONCLUSIONS

Infection of corn with diplodia ear rot (*Diplodia zeae* (Schw.) Lév.) or fusarium ear rot (*Fusarium moniliforme* Sheldon) definitely impairs the digestibility of the contained nitrogen and energy, and the nutritive value of the protein and energy for the promotion of growth in the rat. The damage done by the fusarium organism may be more severe than the damage done by the diplodia organism.

The chemical composition of corn undergoes definite changes as the result of infection with these molds, prominent among which is a marked loss in ether-extractable constituents.

A sample of corn containing as much as 53 percent of kernels damaged by *Gibberella zeae* (Schw.) Petch. [*G. saubinetii* (Mont.) Sacc.] is extremely toxic to young albino rats.

LITERATURE CITED

(1) CHRISTENSEN, J. J., and KERNKAMP, H. C. H.
1936. STUDIES ON THE TOXICITY OF BLIGHTED BARLEY TO SWINE. Minn. Agr. Expt. Sta. Tech. Bul. 113, 28 pp., illus.

(2) CLARK, GEORGE L., and GRING, JOHN L.
1937. CAROTENOIDS IN YELLOW CORN. QUANTITATIVE ESTIMATION FROM MODIFIED ABSORPTION SPECTRA MEASUREMENTS OF CAROTENOIDS IN YELLOW CORN OF DIFFERENT VARIETIES AND QUALITIES. Indus. and Engin. Chem., Analyt. Ed. 9: 271–274.

(3) DUNGAN, G. H., HOLBERT, J. R., MUMM, W. J., BIGGER, J. H., and LANG, A. L.
1935. ILLINOIS CORN PERFORMANCE TESTS. RESULTS FOR 1934. Ill. Agr. Expt. Sta. Bul. 411, pp. 55–88, illus.
(4) GORCICA, H. J., PETERSON, W. H., and STEENBOCK, H.
1935. THE NUTRITIVE VALUE OF FUNGI. III. THE GROWTH OF RATS ON SUPPLEMENTED AND UNSUPPLEMENTED MOLD PROTEINS. Jour. Nutr. 9: 701–714, illus.
(5) KEILHOTZ, F. J., compiler.
1928. SPOILED FEED SHOWN TO BE CAUSE OF PIG TROUBLE. Ill. Agr. Expt. Sta. Ann. Rpt. 41: 144–145.
(6) KOEHLER, BENJAMIN, and HOLBERT, JAMES R.
1930. CORN DISEASES IN ILLINOIS. Ill. Agr. Expt. Sta. Bul. 354, 164 pp., illus.
(7) RABAK, FRANK.
1920. THE EFFECT OF MOLD UPON THE OIL IN CORN. Jour. Indus. and Engin. Chem. 12: 46–48.
(8) RONK, S. E., and CARRICK, C. W.
1931. FEEDING MOLDY CORN TO YOUNG CHICKENS. Poultry Sci. 10: 236–244, illus.
(9) SCHWARTE, L. H.
1938. MOLDY CORN POISONING IN HORSES. Amer. Vet. Med. Assoc. Jour. (n. s. 45) 92: 152–158, illus.
(10) SKINNER, C. E.
1934. THE SYNTHESIS OF AROMATIC AMINO ACIDS FROM INORGANIC NITROGEN BY MOLDS AND THE VALUE OF MOLD PROTEINS IN DIETS. Jour. Bact. 28: 95–106, illus.
(11) TSITOVICH, I. S., and LEVINSON, M. S.
1935. THE TOXIC PROPERTIES OF INDIAN CORN INFECTED WITH FUSARIUM. Problems of Nutrition, Moscow, 4 (1): 85–97. [Abstract in Nutr. Abs. and Revs. 5: 555.]
(12) ZELENY, LAWRENCE, and COLEMAN, D. A.
1939. THE CHEMICAL DETERMINATION OF SOUNDNESS IN CORN. U. S. Dept. Agr. Tech. Bul. 644, 24 pp., illus.

PEA SEED TREATMENT WITH CHEMICAL DUSTS [1]

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INTRODUCTION

Among the first applications of organic mercury dusts to pea seed were those made by Jones (*7*)[3] for the control of seed-borne pathogens, particularly *Ascochyta* spp. It became evident from these investigations that the protection of the seed from decay by organisms in the soil was also an important phase of treatment. The value of certain organic mercury compounds for this purpose was demonstrated by the same investigator (*8*) and by Haenseler (*4*). The former found that germination was reduced markedly when the soil was watered heavily immediately after sowing, particularly with the wrinkled, seeded varieties with sugary cotyledons, as compared with smooth-seeded varieties with starchy cotyledons. Seed treatment offset to a certain degree the detrimental effect of high soil moisture, and in the case of the smooth-seeded variety, Alaska, a good stand was secured with treated seed regardless of the soil moisture and soil temperature. Hull (*6*), in England, noted that high soil moisture reduced emergence owing to the activity of soil organisms on the seeds. He also found that the smooth-seeded varieties, Pilot and Alaska, were less liable to injury than Gradus, a wrinkle-seeded variety, and that organic mercury dusts were helpful in increasing emergence. Brett et al. (*2*), also in England, found seed treatment beneficial to the stand in early-spring sowings, the benefits decreasing as the sowings were delayed. A study in the same country by Padwick (*10*) of the causal agents of pea-seed rotting in the soil showed that a large number of organisms not otherwise pathogenic on peas were involved in this complex disease and that the foot rot organisms such as *Ascochyta pisi* Lib. and *Mycosphaerella pinodes* (Berk. & Blox.) Stone were apparently of little importance in seed rotting.

Following the earlier use of organic mercury dusts other materials were tested. Horsfall (*5*) introduced the use of red copper oxide (Cu_2O), particularly for the variety Surprise. This compound was recommended by Anderson et al. (*1*) in Illinois, by Cook (*3*) in Virginia, and by Ogilvie and Hickman (*9*) in England. Zinc oxide when tried by Cook (*3*) tended to stunt the plants after germination, while Ogilvie and Hickman (*9*) reported it to be less effective than red copper oxide.

The present paper is a report of seed-treatment experiments with canning peas (*Pisum sativum* L.) in Wisconsin during a 4-year period from 1936 to 1939, inclusive, particularly with red copper oxide and 2-percent Ceresan.

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[2] The writers are indebted to Walter Virgin, William Carlsen, John McLean, and Lawrence Plzak, all of whom have assisted in certain phases of this work.
[3] Italic numbers in parentheses refer to Literature Cited, p. 146.

METHODS AND MATERIALS

During 1936 and 1937 tests were carried out on Colby silt loam at the branch experiment station at Marshfield, Wis. Seed was dusted with red copper oxide and graphite according to manufacturers' recommendations and planted in a grain drill in strips 4 feet wide and 16 rods long. Three plots of each treatment were planted on May 15, 1936; two plots of each on May 25, 1937. Stand estimates were made about 4 weeks after planting by taking the counts of plants in square-yard areas at several random locations in each plot.

In 1938 and 1939, single-row plots of 110 or 150 seeds per row were used. The individual row lots were dusted in separate packets and planted at approximately 10 seeds per foot of row. At the time of planting, the hands were dusted with the material used on the seed in order to avoid removal of dust from the seeds during the planting operation. All excess dust was left in each envelope. Four row-plots of each lot of seed were used for each treatment arranged in random order. Counts of the complete stand in each were made about 4 weeks after planting. In addition to the Marshfield tests in 1938 (sown May 2), the same plot design was repeated in an early (April 25) and a late (June 3) planting on Miami silt loam at the branch experiment station at Sturgeon Bay, Wis., and in an early planting (May 3) on the same soil type on the university farm at Madison, Wis. In 1939 plantings were made on May 9 at Marshfield, on the same date at Sturgeon Bay, and on May 13 at Madison.

The pea seed used was secured from the branch experiment station at Sturgeon Bay. The red copper oxide was provided by Röhm and Haas Co. The Ceresan contained 2 percent of ethyl mercuric chloride and was provided by the Bayer-Semesan Co.

For comparative purposes the rainfall record was used for the period beginning 3 days before sowing and extending for 11 days beyond sowing for each location. Significance of difference in all cases was checked by the analysis of variance method.

EXPERIMENTAL RESULTS

The stand counts at Marshfield for 1936 and 1937 are given in table 1. At this location no significant increase in stand was secured with either the smooth-seeded Alaska or the four wrinkle-seeded varieties. No detrimental effect was apparent except a temporary slight retardation in vine growth in 1937 with all varieties except Wisconsin Penin. The soil moisture at the time of sowing was lowest in 1937.

In table 2 are the stand counts from all plots in 1938 and 1939. It will be noted that two additional locations were added. Again no benefits in stand were secured in 1938 with the Alaska seed from either treatment. At Madison the only significant increase was with red copper oxide on one variety, Wisconsin Perfection. At Marshfield the oxide was beneficial on all three wrinkle-seeded varieties, whereas with Ceresan no significant increases in stand were secured. At Sturgeon Bay significant increases were noted at both planting dates with each compound in all wrinkle-seeded varieties except Merit. In a majority of cases the increase in stand was greater with red copper oxide than with Ceresan. There was no clear-cut correlation between soil moisture as indicated by the precipitation records and the effectiveness of treatment.

TABLE 1.—*The effect of red copper oxide treatment on stand of pea varieties planted at the Marshfield Experiment Station, 1936 and 1937*

Variety	Year of test	Rainfall [1]	Stand counts (plants per square yard)		
			Untreated	Treated	Difference required for significance between treatments (1:19)
		Inches	*Number*	*Number*	
Alaska	1936	1.32	83.8	81.3	10.8
	1937	.56	99.8	90.0	24.0
Wisconsin Early Sweet	1936	1.32	130.5	125.8	10.8
Surprise	1937	.56	106.8	103.8	24.0
Wisconsin Perfection	1936	1.32	72.2	77.9	10.8
Wisconsin Penin	1937	.56	73.0	74.0	24.0

[1] For the period inclusive of 3 days before and 11 days after sowing.

TABLE 2.—*The effect of seed treatment on stand of pea varieties in 1938 and 1939*

1938 EXPERIMENTS

Variety	Location of trial	Date of sowing	Rainfall [1]	Stand in the various treatments			Difference required for significance (1:19)	Significant (+) or insignificant (−) increase over untreated	
				Untreated	2-percent Ceresan	Red copper oxide		Ceresan	CuO
			Inches	*Percent*	*Percent*	*Percent*	*Percent*		
Alaska	Marshfield	May 2	2.51	78.7	76.0	78.0	7.3	−	−
	Madison	May 3	.66	97.3	98.0	98.7	6.0	−	−
	Sturgeon Bay	Apr. 25	2.34	90.0	91.8	92.7	6.4	−	−
	do	June 3	.44	85.5	83.6	91.8	7.3	−	−
Wisconsin Early Sweet	Marshfield	May 2	2.51	58.0	57.3	65.3	7.3	−	+
	Madison	May 3	.66	91.3	92.7	96.0	6.0	−	−
	Sturgeon Bay	Apr. 25	2.34	70.0	80.0	90.0	6.4	+	+
	do	June 3	.44	55.5	78.2	89.1	7.3	+	+
Wisconsin Perfection	Marshfield	May 2	2.51	32.7	38.0	44.0	7.3	−	+
	Madison	May 3	.66	65.2	68.0	74.0	6.0	−	+
	Sturgeon Bay	Apr. 25	2.34	40.0	54.5	65.5	6.4	+	+
	do	June 3	.44	39.1	67.3	62.7	7.3	+	+
Wisconsin Penin	Marshfield	May 2	2.51	57.3	62.7	66.7	7.3	−	+
	Madison	May 3	.66	85.3	86.7	88.0	6.0	−	−
	Sturgeon Bay	Apr. 25	2.34	63.6	74.5	79.1	6.4	+	+
	do	June 3	.44	66.4	74.5	80.9	7.3	+	+
Merit	Sturgeon Bay	June 3	.44	81.8	87.3	89.3	7.3	−	+
Canner King	do	do	.44	67.3	79.1	85.5	7.3	+	+
Surprise	do	do	.44	46.4	65.5	75.5	7.3	+	+

1939 EXPERIMENTS

Alaska	Marshfield	May 9	.00	95.2	98.5	97.3	5.0	−	−
	Madison	May 13	.20	91.9	95.9	90.7	8.0	−	−
	Sturgeon Bay	May 9	1.26	95.2	97.9	98.0	9.3	−	−
Wisconsin Early Sweet	Marshfield	May 9	.25	91.0	91.9	93.2	5.0	−	−
	Madison	May 13	.20	85.3	82.5	85.2	8.0	−	−
	Sturgeon Bay	May 9	1.26	33.9	55.3	93.5	9.3	+	+
Wisconsin Perfection	Marshfield	May 9	.25	82.7	80.9	81.9	5.0	−	−
	Madison	May 13	.20	84.9	87.3	90.0	8.0	−	−
	Sturgeon Bay	May 9	1.26	65.2	72.5	87.0	9.3	−	+
Wisconsin Penin	Marshfield	May 9	.25	73.3	78.3	75.9	5.0	+	−
	Madison	May 13	.20	79.3	77.9	77.7	8.0	−	−
	Sturgeon Bay	May 9	1.26	57.9	61.7	83.5	9.3	−	+

[1] For the period inclusive of 3 days before and 11 days after sowing.

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In 1939 the two treatments were again used at each of the three locations of 1938. At Marshfield and Madison the season was very dry at the time of sowing. No increases of consequence, with one exception, were secured at either of these stations (table 2). At Sturgeon Bay the Alaska was again not influenced by treatment, while red copper oxide caused significant increases in stand for the three wrinkle-seeded varieties. The Ceresan treatment was effective in improving the stand of only one variety at Sturgeon Bay—Wisconsin Early Sweet—but in this case its effect was much less than that of red copper oxide.

DISCUSSION AND SUMMARY

The results of 4 years' trials with treatment of pea seed in Wisconsin show clearly that the benefits to be derived will vary with the season and location. All the fields used had been subjected to rather intensive pea production previous to the experiments. In accord with the findings already cited from England, the conditions which bring about stand reduction of sugary-cotyledoned forms are not detrimental to the starch-seeded Alaska, and consequently seed treatment was of no value to this variety at any time during the 4-year period.

In the soil of relatively high water-holding capacity at Marshfield significant increases from treatment were secured in only 1 out of 4 years. This was in the year of heaviest precipitation at that station during the planting period. It is important to note, however, that in that season (1938) only red copper oxide was beneficial and the increases in stand, although significant, were decidedly lower than those of the same seed samples in treated plots at other locations. It is obvious that at Marshfield, in this season, treatment did not correct adequately the "poor stand" difficulty.

The Sturgeon Bay soil, although of the same classification as that of Madison, benefited more consistently from pea-seed treatment than did the latter. In the early plantings of 1938 and 1939 it was in each case the moister soil. The early and late sowings at Sturgeon Bay in 1938, however, both showed benefits of treatment while the rainfall during the first sowing period was 2.34 inches as compared with 0.44 inch during the second.

It may be said, therefore, that the conditions which favor cotyledon decay during germination occur periodically but not necessarily consistently under average Wisconsin conditions. They do not seem to affect the stand of starchy-cotyledoned forms, but may reduce the stands of the sugary-seeded varieties. Red copper oxide is a safe and beneficial protective treatment for all varieties of the latter group. Ceresan, while sometimes beneficial, was not so effective as the oxide in these trials.

LITERATURE CITED

(1) Anderson, H. W., Kadow, K. J., and Hopperstead, S. L.
1937. The evaluation of some cuprous oxides recommended as seed-treatment products for the control of damping off. Phytopathology 27: 575–587, illus.

(2) Brett, C. C., Dillon Weston, W. A. R., and Booer, J. R.
1937. Seed disinfection. III. Experiments on the germination of peas. Seed protection by the use of disinfectant dusts containing mercury. Jour. Agr. Sci. [England] 27: 53–66, illus.

(3) Cook, Harold T.
1937. spinach and cabbage seed treatment. Va. Truck Expt. Sta. Bul. 96, pp. [1491]–1510, illus.
(4) Haenseler, C. M.
1928. effect of organic mercury seed treatments on germination and yield of peas. N. J. Agr. Expt. Sta. Ann. Rpt. (1927) 48: 232–238.
(5) Horsfall, J. G.
1936. drilling pea seed dusted with red copper oxide. Canner 82 (4): 32, 34.
(6) Hull, R.
1937. effect of environmental conditions, and more particularly of soil moisture upon the emergence of peas. Ann. Appl. Biol. 24: 681–689, illus.
(7) Jones, Leon K.
1927. studies of the nature and control of blight, leaf and pod spot, and footrot of peas caused by species of ascochyta. N. Y. State Agr. Expt. Sta. Bul. 547, 46 pp., illus.
(8) ———
1931. factors influencing the effectiveness of organic mercury dusts in pea-seed treatment. Jour. Agr. Res. 42: 25–33, illus.
(9) Ogilvie, L., and Hickman, C. J.
1938. cuprous oxide and zinc oxide as seed protectants. Gard. Chron. (3) 103: 79–80, illus.
(10) Padwick, G. W.
1938. complex fungal rotting of pea seeds. Ann. Appl. Biol. 25: 100–114, illus.

THE EFFECT OF SOME MINERAL NUTRIENTS ON THE DEVELOPMENT OF CLUBROOT OF CRUCIFERS [1]

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INTRODUCTION

Many workers (*2, 3, 7, 10, 11, 12, 13, 15, 16*)[3] have demonstrated that varying the nutrition of a plant has a decided effect on disease development. In the majority of these studies nitrogen, potassium, and phosphorus were the elements under investigation. The specific response appeared to vary with the degree of plant resistance, with the parasite involved, and with the nutrients supplied. Petri (*7*) observed that, in general, nitrogenous dressings aggravate susceptibility, whereas phosphatic and potassic applications reduce it. In regard to the variety or strain of the host the findings of Gassner and Hassebrauk (*3*) with several rusts of wheat, barley, and oats illustrate the general case. Partially resistant hosts proved to be the most suitable material for nutritional experiments. If the plants were either highly resistant or immune, varying the proportion and amount of salts in the culture solution did not change the disease reaction, whereas if extremely susceptible plants were employed the disease attack was so severe that the nutritional effects were masked.

The possible influence of sulfur oils in cruciferous plants upon the development of clubroot (*Plasmodiophora brassicae* Wor.) was first suggested by Rochlin (*9*). The purpose of the present investigation was to vary the oil content of the host through controlled nutrition and to study the consequent effect upon the development of the disease. The mustard oils present in crucifers all contain sulfur and nitrogen while their glycosides also contain potassium. These nutrient elements were selected for study.

METHODS

In these experiments Shogoin, Cowhorn, and Purple Top Milan varieties of turnip (*Brassica rapa* L.), two strains of black mustard (*B. nigra* Koch) and Jersey Queen variety of cabbage (*B. oleracea* var. *capitata* L.) were used. The average percentages of clubroot-infected plants from several greenhouse trials of the above lots of crucifers

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[3] Italic numbers in parentheses refer to Literature Cited, p. 159.

grown on naturally infested soil collected in southern Wisconsin are as follows:

	Percent of plants diseased
Strain No. 14 black mustard	85
Strain No. 10 black mustard	5
Shogoin turnip	100
Cowhorn turnip	40
Purple Top Milan turnip	0
Jersey Queen cabbage	100

As already pointed out (*18*) it is essential in greenhouse tests with clubroot to keep the soil reaction fairly acid; the soil in these tests was maintained at pH 6.0 to 6.5.

The foregoing forms were selected from collections previously made by Walker (*17*, *18*) as representing highly resistant (No. 10) and highly susceptible (No. 14) strains of black mustard, and completely susceptible (Shogoin) and completely immune (Purple Top Milan) varieties of turnip. The Cowhorn variety of turnip was selected because it consistently showed an intermediate percentage of infected plants and was undoubtedly heterozygous for the respective characters which had been completely or almost completely fixed in the other two varieties. Jersey Queen cabbage is representative of another form, all varieties of which are very susceptible to *Plasmodiophora brassicae*.

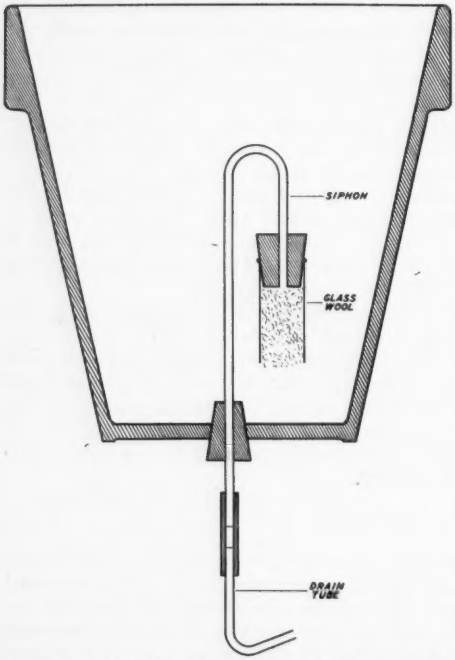

FIGURE 1.—Siphon drain for sand-culture pots. The bend in the 5-mm.-bore glass tubing is 2½ inches below the top of the pot. The 2-inch piece of 20-mm. glass tubing filled with glass wool serves to prevent sand from clogging the drain.

Heavily varnished (*6*), 8-inch clay pots were equipped with a modification of Tharp's automatic siphon (*14*) (fig. 1) which allowed the sand to become saturated about every 36 hours under a supply system to be described later. These pots were filled to within one-half of an inch of the top with fine white silica sand which had been washed several times in hot water and rinsed three or four times with distilled water.

Nutrients were supplied by a modification of the continuous-flow apparatus described by Allison and Shive (*1*). From 10-gallon reservoirs solutions flowed by siphon and gravity through 12-mm.

tubing to a 12-inch length of 0.3-mm.-bore capillary tubing, which was found to deliver approximately 1 liter per day. One section of a Petri dish was embedded with open side downward at one-half of an inch below the surface of the sand in the pot to help distribute the nutrients. To prevent algal growth the reservoirs were covered and all glass which was exposed to the sunlight was painted black. Thirty inches above the bench a framework was constructed to support the capillaries. Compressed air was allowed to bubble through the solution in the supply vessels in order to aerate and stir the culture liquid.

Modifications of Hoagland's nutrient solution were used. In table 1 are given the respective amounts of chemicals (reagent grade) which were diluted with distilled water to make the different culture liquids. Since infection by *Plasmodiophora brassicae* is favored by an acid medium, enough hydrochloric acid was added to keep the sand at about pH 6.5. The small amount of sodium chloride was included in each series in order to eliminate any possible differential effect from the presence or absence of sodium in the various culture solutions.

TABLE 1.—*Composition of culture solutions used in the study of the effect of host nutrition on clubroot development*

Composition and concentration (moles per liter) of stock solution	Amounts mixed and brought to 18 liters to make up the various solutions					
	Complete	−S	+N	−N	+K	−K
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
$Ca(NO_3)_2.4H_2O$, 1.0	9.0	9.0	9.0		9.0	9.0
$CaCl_2.6H_2O$, 1.0				9.0		
KNO_3, 1.0	9.0	9.0	9.0		9.0	
KCl, 1.0				9.0	3.0	
KH_2PO_4, 1.0	1.8	1.8	1.8	1.8	1.8	
NaCl, 1.0	1.0	1.0	1.0	1.0	1.0	1.0
$NaNO_3$, 1.0			4.0	2.0		9.0
$NaH_2PO_4.H_2O$, 1.0						1.8
$MgSO_4.7H_2O$, 1.0	3.6		3.6	3.6	3.6	3.6
$MgCl_2.6H_2O$, 1.0		3.6				
HCl, .1	9.0	9.0	9.0	9.0	9.0	9.0
A–Z [1]	1.8	1.8	1.8	1.8	1.8	1.8

[1] The A–Z stock solution was composed of the following, made up to 1,000 cc. with distilled water: H_3BO_3, 2.818 gm.; $CuCl_2.2H_2O$, 0.040 gm.; $ZnCl_2$, 0.030 gm.; $MnCl_2.4H_2O$, 0.390 gm.; and $FeCl_3.6H_2O$, 5.000 gm.

The proportion of the salts was so arranged as to secure: (1) in the minus-element series, symptoms of deficiency not severe enough to cause death of the plant; (2) in the complete series, moderately growing plants; (3) in the plus-element series, plants showing distinct vegetative stimulation from an extra supply of nitrogen or potassium but no sign of excess symptoms.

Seed was sown in each pot, and after approximately 2 weeks the seedlings were thinned to about 25 plants per pot. To make the inoculum, cabbage clubs were washed and run through a food chopper and, after distilled water was added to the macerate, the suspension was filtered through cheesecloth. The liquid was then centrifuged and when the supernatant liquid had been decanted, more distilled water was added to the residue and the suspension shaken well. The washing process was repeated several times to remove all possible soluble

salts. The resultant gummy spore mass was diluted with enough water, adjusted to pH 6.5 with hydrochloric acid, to provide 100 cc. for each pot. At the first true-leaf stage and 2 weeks later the plants were inoculated with the spore suspension applied to the surface of the sand with a pipette. Plants were pulled and examined for clubroot about 10 weeks after the seed was sown.

Replicate pots were separated by pots of other forms and the arrangement changed each time the experiment was repeated to eliminate variations arising from environmental differences. There seemed to be no appreciable effect from the different positions. All the nitrogen and potassium experiments were run simultaneously in the same greenhouse and one sulfur series was included with these groups; thus the different treatments may be compared to a certain extent. Other trials with sulfur had been made earlier. The composited results for each series represent the totals from two or more experiments unless otherwise designated.

Since McMurtrey (*4*) has described the symptoms on many plants resulting from nutrient deficiency, the appearance of plants grown without sulfur, nitrogen, or potassium will not be described in great detail.

EXPERIMENTAL RESULTS

EFFECT OF SULFUR

Symptoms of sulphur deficiency were quite severe after 4 to 6 weeks. In mustard there was a pronounced difference in pungency between the complete and the minus-sulfur-fed plants when the leaves were tasted. Chemical analysis [4] of one series of sulfur-deficient plants indicated sulfur oils to be practically absent. The sulfur-deficient plants were slightly less green in color and quite stunted. Sometimes a red pigment developed at the leaf margins and near the stem tip. On cabbage there was, in addition to the external symptoms described for mustard, a slight yellowing at the leaf margins and a distinct mottle in the blade. Shogoin and Cowhorn turnips tended to turn more yellow at the leaf margin and show less mottling, while Purple Top Milan turnip was in an intermediate position with regard to symptoms between Shogoin turnip and cabbage.

When the plants were removed and examined for clubbing, three fairly distinct types of diseased roots were noticed. One was the normal, relatively smooth club which occurred most abundantly on the susceptible plants in sulfur-deficient nutrient (fig. 2). Another, found most frequently on resistant plants, was a small gall 1 or 2 mm. in diameter appearing usually, but not always, at the base of a branch root (fig. 3). The third type of overgrowth, seemed to be composed of a great many enlarged galls. These were grouped in the same class as clubs. In some cases these enlargements, which were also more generally prevalent on the resistant strains, became so numerous that they coalesced to form a club very irregular in outline. Figure 4 illustrates this range of symptoms on Cowhorn turnip, a variety intermediate in resistance to clubroot.

In table 2 are the composite results of all the nutrition experiments. The total number of plants and the percentage without visible symp-

[4] The author is indebted to Mark Stahmann of the Department of Biochemistry, University of Wisconsin for making this analysis.

toms, with galls, and with clubs are presented. Sulfur deficiency brought about an increase in the percentage of clubbed plants in both susceptible and resistant [5] strains, the greatest differences being in the resistant hosts. The number of plants with galls in the resistant strains was augmented by the treatment, but, although this type of overgrowth was of rather rare occurrence in the susceptible group, it is to be noted that the trend was always in the direction of more galls in the complete solution. The point is significant, since these en-

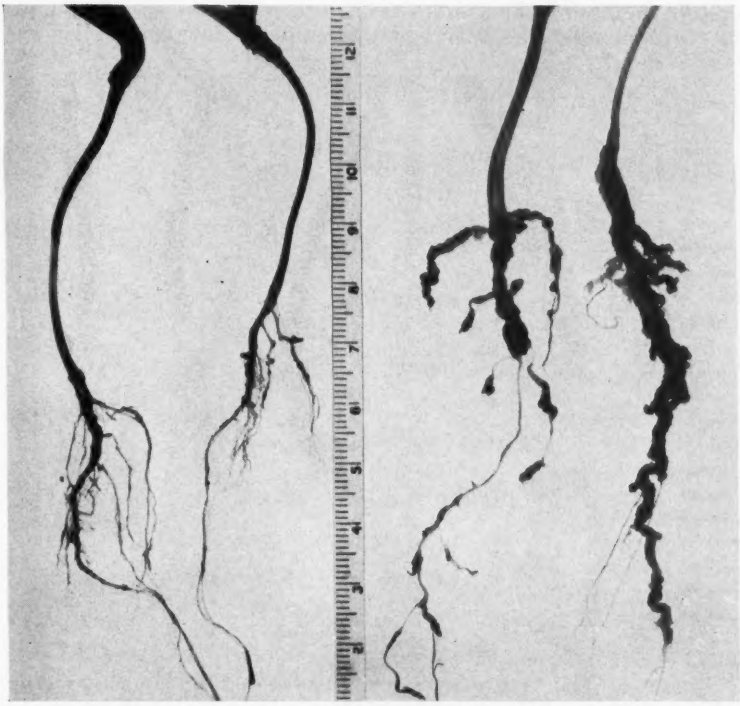

FIGURE 2.—The usual type of club on plants grown in infested sand supplied with artificial nutrient (Shogoin turnip). Scale is in millimeters.

largements were observed most commonly in resistant black mustard in the preliminary trials in soil cited above.

At no time did any evidence of clubroot infection appear on the immune Purple Top Milan turnip.

In one case a few healthy-appearing mustard plants and some mustard plants with galls were thoroughly washed to remove adhering spores of *Plasmodiophora brassicae*. One lot each of healthy and gall-bearing mustard was replanted in sterile sand supplied with a full nutrient solution. A group of each was also set in sand furnished

[5] Unless otherwise designated, the term "resistant" has been used to indicate varieties in which only a few plants show macroscopically visible disease symptoms. Immune strains are ones in which no plants have macroscopic disease symptoms.

with a sulfur-deficient solution. After 1 month there was little change in root symptoms within the group in the complete nutrient solution. In the minus-sulfur set, however, some of the galls had developed into clubs and in one or two cases galls were found on plants which had previously appeared to be healthy. The formation of galls on apparently healthy plants and the development of galls into clubs on diseased plants during this period of deficient nutrition would seem to indicate that some plants in both groups had been infected previous to transplanting. After being transferred to new culture conditions, the plants were evidently able to hold the fungus in abeyance when the nutrition was complete, but when sulfur was

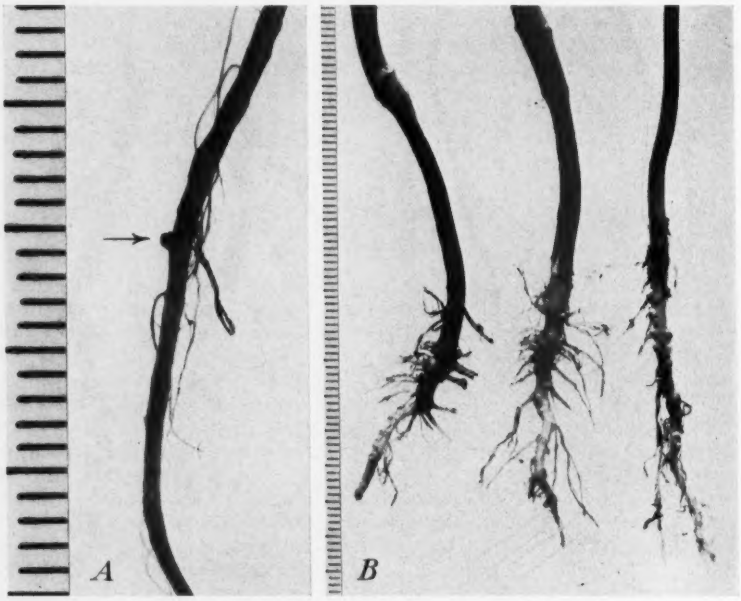

FIGURE 3.—The "gall" type of clubroot symptom on Cowhorn turnip (*A*) and on strain No. 10 of black mustard (*B*). Scale is in millimeters.

absent, more pronounced root symptoms appeared as a result of further invasion by *P. brassicae*. These observations, although made on a small number of plants, seem to conform to those cited above in which the minus-sulfur nutrition tended to increase the percentage of clubs that were normal in size and shape.

EFFECT OF NITROGEN

Three to four weeks after the plants appeared above the surface of the sand, nitrogen-deficiency symptoms became evident. In all varieties the cotyledons turned yellow more quickly on the nitrogen-deficient plants than on those grown with complete nutrients. The leaves were smaller and more yellow in color and a red to purple pigment appeared on the stems usually 2 to 3 weeks before the plants were removed for examination. A heavier coating of bloom devel-

oped on nitrogen-deficient cabbages than on the plants in the complete nutrient.

In contrast to the sulfur-deficiency symptoms, the characteristic odor and taste of the sulfur oils in the nitrogen-deficient plants ap-

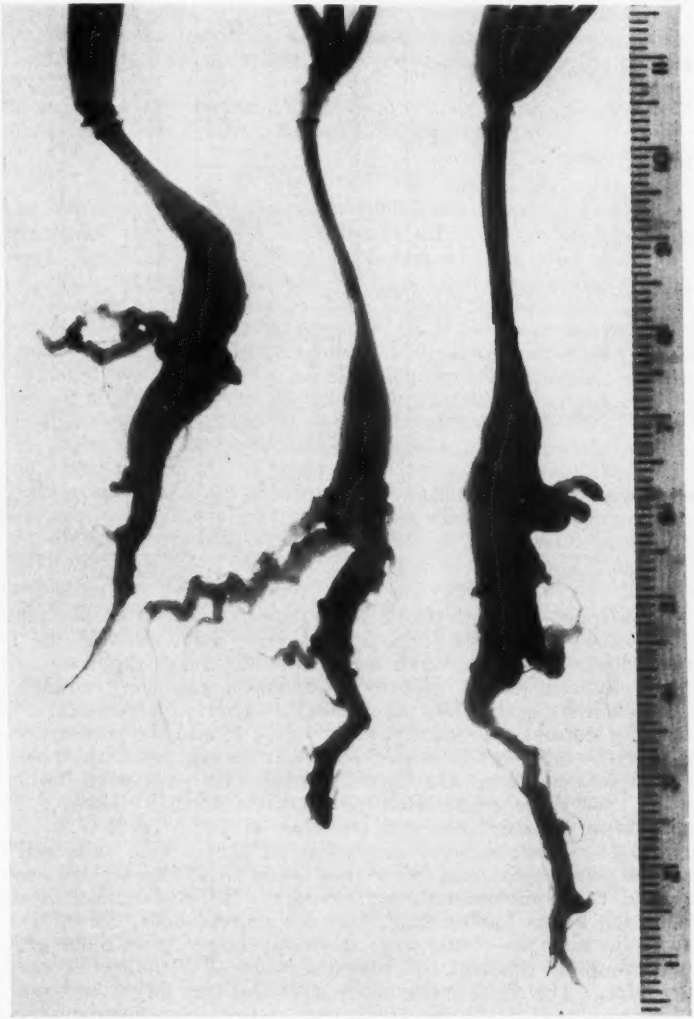

FIGURE 4.—Range of clubbing found on Cowhorn turnip when grown in infested sand supplied with artificial nutrients. Scale is in millimeters.

peared to be just as strong as in the plants in the complete solution. By this crude test there also seemed to be slightly less mustard oil in the plus-nitrogen plants than in the check plants, but the oil was present in sufficient quantity to make the pungent taste noticeable.

TABLE 2.—*The effect of host nutrition on the development of clubroot in certain forms of crucifers*

Variety	Nutrient solution [1]	Total plants	Healthy	Galls [2]	Clubs
		Number	*Percent*	*Percent*	*Percent*
No. 14, black mustard	Complete	330	9.1	1.2	89.7
	−S	130	3.1	.8	96.1
	+N	258	4.6	.4	95.0
	−N	243	1.2	.0	98.8
	+K	71	5.6	.0	94.4
	−K	140	69.3	2.1	28.6
No. 10, black mustard	Complete	333	89.8	3.6	6.6
	−S	219	74.0	10.9	15.1
	+N	236	78.4	9.3	12.3
	−N	223	76.7	7.6	15.7
	+K	74	89.2	2.7	8.1
	−K	159	93.7	1.9	4.4
Shogoin turnip	Complete	344	26.2	2.3	71.5
	−S	158	6.9	1.3	91.8
	+N	192	10.4	2.1	87.5
	−N	181	13.8	2.2	87.5
	+K	69	14.5	.0	85.5
	−K	215	82.3	.0	17.7
Cowhorn turnip	Complete	204	63.7	5.4	30.9
	−S [3]	72	30.6	11.1	58.3
	+N	227	52.9	14.1	33.0
	−N	224	51.3	4.5	44.2
	+K [3]	90	81.1	.0	18.9
	−K	217	96.3	.5	3.2
Purple Top Milan turnip	Complete	125	100.0	.0	.0
	−S	112	100.0	.0	.0
	+N	34	100.0	.0	.0
	−N	29	100.0	.0	.0
	+K	51	100.0	.0	.0
	−K	50	100.0	.0	.0
Jersey Queen cabbage	Complete	328	17.4	1.2	81.4
	−S	137	8.0	.0	92.0
	+N	193	11.4	.5	88.1
	−N	207	7.7	.0	92.3
	+K	62	6.5	1.6	91.9
	−K	190	77.9	2.6	19.5

[1] See table 1. [2] See figure 3. [3] 1 experiment only.

The nitrogen-deficient plants were much smaller than the plants in any other series, yet clubs formed vigorously, most of the few branch roots which appeared as well as the main tap root being entirely hypertrophied. Nitrogen starvation was very similar to sulfur deficiency in its effect on clubroot development (table 2). The symptoms were of the same type (figs. 2, 3, 4) and the percentage of plants with clubs was increased markedly as compared to those in the complete solution. Susceptible plants with galls were fewer in number than those grown with the complete solution; however, gall formation on resistant varieties was more erratic. In No. 10 black mustard there was a larger proportion of plants with galls and in Cowhorn turnip galls were fewer than when these two varieties were furnished the complete nutrient solution. No explanation of this observation seems forthcoming from the present data.

The plus-nitrogen plants were distinctly larger than those grown in the complete nutrient solution and were of a somewhat deeper green color. The clubs in this group were also very large as compared to those produced in the minus-nitrogen and complete-nutrient series. More nitrogen than that furnished in the complete solution aided clubroot development. The increase in clubbing of high-nitrogen plants over those grown with complete nutrients was less in resistant than in the susceptible strains. Where there was increased suc-

culence and rate of growth in the host, induced by an extra supply of nitrogen, a larger percentage of clubs developed than in the complete solution. The positive correlation of vigorous growth of the host and rapid development of the parasite has been observed commonly with other diseases. With respect to the appearance of galls, high nitrogen tended to have the same influence as low sulfur. The proportion of plants with galls in susceptible forms was less than that in the complete solution, while the opposite was true for the resistant strains.

As in the sulfur series, Purple Top Milan turnip proved to be immune regardless of the variation in nitrogen supply.

EFFECT OF POTASSIUM

The first symptom of potassium starvation appeared as a definite yellowing of the foliage 5 to 7 weeks after the cotyledons broke through the sand. The plants were somewhat smaller than those grown with complete nutrients but the reduction in size was not nearly as great as that caused by deficiencies of the other two elements. In 6 to 8 weeks severe necrosis appeared on these lower leaves both at the margin and in spots on the blade. These lower leaves later became dry but did not drop, and gradually the necrosis appeared on leaves higher on the stem. No difference in mustard-oil content could be detected between the plants at any of the potassium levels.

The same types of clubroot symptoms appeared on these plants as on those grown in the other nutrients already described. The largest change in amount of infection was brought about by potassium deficiency (table 2). The reduction in disease development as a result of this deficiency was approximately 60 percent in the susceptible varieties when compared with the percentage of infection of these same varieties furnished a complete supply of nutrients. The amount of reduction seemed to depend on the degree of resistance of the strains used, the greater effect being with the susceptible lines. With No. 10 black mustard where complete-nutrient plants developed 6.6 percent clubs, the percentage was reduced by only 2.2 percent; while with Cowhorn turnip, which was 30.9 percent clubbed in the complete solution, the percentage was reduced by 27.7 percent. The appearance of galls on the susceptible varieties showed no constant variation, but on the resistant strains the percentage of plants with galls tended to decrease with an insufficiency of potassium.

In the plus-potassium series the plants were somewhat larger than those grown in the complete solution and slightly smaller than those in the plus-nitrogen series. With all the varieties except Cowhorn the proportion of plants with clubs was augmented and on all varieties the appearance of galls was decreased as compared with plants in the complete solution.

It should be pointed out that no clubroot symptoms were found on Purple Top Milan turnip in the potassium series.

DISCUSSION

Although it has been reported difficult to secure clubroot on plants in sand culture (*5*) with the methods reported herein, crucifers in artificial nutrient solution were almost as severely attacked as were those grown in heavily infested soil. With some forms the percentage of

infection was higher and with others lower than when plants of the same variety were grown in infested soil. No explanation for this situation seems adequate at the present time.

Corroborating the work of Gassner and Hassebrauk (*3*), the results of these tests indicated that varying nutrition had the most pronounced effect on disease development in hosts which were to a certain degree susceptible, but did not influence resistance in plants such as Purple Top Milan, a variety of turnip immune to Wisconsin collections of the pathogen. Resistance does not seem to be correlated with vegetative vigor. There was a wide difference in the percentage of clubs in the N-, S-, and K-deficient series even though all of the plants showed reduced vigor. These observations are similar to those made on other diseases (*13*, *15*). In contrast to these other cases, however, it was noticed that the enhanced growth which resulted from an extra supply of nitrogen or potassium also promoted the development of clubs, although not as pronouncedly as did the deficiency in sulfur or nitrogen.

Since *Plasmodiophora brassicae* has not been grown in pure culture it is not easy to determine whether the elements used affect the parasite directly. Sulfur and nitrogen at least do not seem to be necessary in abundance for the parasite to act as a virulent pathogen, while the absence of potassium either decreases penetration or affects the host in such a way as to be unfavorable to *P. brassicae*. In relation to this latter element, it is of interest to note the analysis of clubbed and healthy cabbage roots made by Reed (*8*). He found that although the ash constituents were present in appreciably greater amounts in diseased than in healthy tissue, the greatest increase of any single element was in the case of potassium. In view of this analysis and the fact that potassium deficiency markedly decreased the number of clubs, it may be that the element is necessary for the growth processes of the parasite as well as those of the host.

Since decreasing the amount of nitrogen without appreciably lowering the mustard-oil content increased clubroot infection to about the same degree as eliminating sulfur in the culture solution, sulfur oils do not appear to be necessary to the host in preventing or retarding development of *Plasmodiophora brassicae*. Upon the basis of the data obtained in the present investigation it is not possible to say why pronounced nitrogen and potassium deficiency should not greatly lower the amount of sulfur oil.

Plasmodiophora brassicae subsists in living host cells either directly upon the host protoplasm or upon products produced or absorbed by the host cell. In either case its existence in the vegetative stage is dependent upon the living plant. As a result any marked disturbance of host metabolism by mineral deficiency might affect the relationship with the parasite in such a way as to increase the competition for elaborated food and perhaps for the mineral nutrients. Sulfur, nitrogen, and potassium being essential elements, are necessary not only because they are constituents of certain indispensable compounds, but also because they influence the formation of requisite substances in which they are not present—notably the carbohydrates. Thus decreased resistance may be due to the absence of certain compounds, to the accumulation of others, or to metabolic processes which are as yet unknown. Immunity such as that occurring in Purple Top

Milan turnip must be even more closely related to the host protoplasm since drastic changes in host metabolism did not alter the ability of the plant to prevent clubroot development in its tissue.

SUMMARY

Methods have been reported whereby clubroot infection was secured in sand culture. The percentage of diseased plants was comparable to that obtained in infested, acid soil. Upon the basis of symptoms, plants were grouped into three classes: (1) Those with no symptoms, (2) those with clubs, (3) those with galls. The clubs were of the usual type mentioned by other workers. The galls were small spherical overgrowths, 1 or 2 mm. in diameter, appearing usually, but not always, at the base of a branch root and being more prevalent on the resistant varieties.

The effect of sulfur, nitrogen, and potassium nutrition on the development of clubroot in susceptible, resistant, and immune strains of crucifers was studied. The proportion of nutrients in the different experiments was adjusted so as to produce: (1) Plants showing deficiency symptoms, (2) plants growing moderately, and (3) plants having pronounced vegetative vigor resulting from an extra supply of nitrogen and potassium.

The percentage of susceptible plants having clubs was in general increased slightly over that in the complete solution by an abundance of potassium, more by an abundance of nitrogen, and most by the absence of sulfur or nitrogen. The percentage was decreased markedly in plants deficient in potassium. A smaller proportion of plants of susceptible strains developed galls when grown in nutrient solutions in which sulfur or nitrogen was withheld than was the case with resistant strains.

The percentage of resistant plants having clubs was increased somewhat by high nitrogen, but it was greatest in the cases of sulfur or nitrogen deficiency. With an extra amount of potassium the results were inconclusive. The proportion of plants with clubs was decreased definitely by lack of potassium. The formation of galls was usually increased by the absence of sulfur or by high nitrogen and was generally decreased by high or low potassium, but the effect of the other nutrition series on the presence of these overgrowths was not conclusive.

No signs of clubroot appeared regardless of the variation in nutrient supply on the immune variety of turnip, Purple Top Milan.

Deficiency of sulfur lowered the sulfur-oil content greatly in the crucifer varieties while nitrogen starvation did not. On a given variety, the number of plants with clubs was increased to approximately the same extent by the absence of either of these elements. From these observations it would appear that the sulfur oils are not essential in enabling the host to prevent or retard clubroot development in its tissues.

LITERATURE CITED

(1) Allison, R. V., and Shive, J. W.
1923. Studies on the relation of aeration and continuous renewal of nutrient soi ution to the growth of soybeans in artificial culture. Amer. Jour. Bot. 10: 554–566, illus.

(2) Fisher, Paul Lewis.
1935. physiological studies on the pathogenicity of fusarium lycopersici sacc. for the tomato plant. Md. Agr. Expt. Sta. Bul. 374, pp. 261–281, illus.
(3) Gassner, G., and Hassebrauk, K.
1931. untersuchungen über die beziehungen zwischen mineralsalzernährung und verhalten der getreidepflanzen gegen rost. Phytopath. Ztschr. 3: [535]–617, illus.
(4) McMurtrey, J. E., Jr.
1938. distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev. 4: 183–203, illus.
(5) New Jersey Agricultural Experiment Station.
1937. cabbage club root. N. J. Agr. Expt. Sta. Ann. Rpt. 58: 80.
(6) Norem W. Luther.
1936. mineral nutrition and seasonal growth of ageratum in sand cultures with auto-irrigation. Amer. Jour. Bot. 23: 545–555, illus.
(7) Petri, L.
1930. la nutrizione minerale delle piante in rapporto alla predisposizione o alla resistenza di queste a cause patogene. R. Staz. Patol. Veg. Bol. (n. s.) 10: 121–152. [Abstract in Rev. Appl. Mycol. 10: 200. 1931.]
(8) Reed, Howard S.
1911. the effect of the club root disease upon the ash constituents of the cabbage root. Phytopathology 1: 159–163.
(9) Rochlin, Emilia.
1933. zur frage der widerstandsfähigkeit der cruciferen gegen die kohlhernie (plasmodiophora brassicae wor.). Phytopath. Ztschr. 5: [381]–406, illus.
(10) Schaffnit, E., and Volk, A.
1927. über den einfluss der ernährung auf die empfänglichkeit der pflanzen für parasiten. (teil 1.) Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 3: 1–145, illus.
(11) Spencer, Ernest L.
1935. effect of nitrogen supply on host susceptibility to virus infection. Phytopathology 25: 178–191, illus.
(12) ———
1935. influence of phosphorus and potassium supply on host susceptibility to yellow tobacco mosaic infection. Phytopathology 25: 493–502, illus.
(13) ——— and McNew, George L.
1938. the influence of mineral nutrition on the reaction of sweet-corn seedlings to phytomonas stewarti. Phytopathology 28: 213–223, illus.
(14) Tharp, W. H.
1938. a sand-nutrient infection technique for the study of fusarium wilt of cotton. Phytopathology 28: 206–209, illus.
(15) Trelease, Sam F., and Trelease, Helen M.
1928. susceptibility of wheat to mildew as influenced by salt nutrition. Torrey Bot. Club Bul. 55: 41–68, illus.
(16) Volk, A.
1931. beiträge zur kenntnis der wechselbeziehungen zwischen kulturpflanzen, ihren parasiten und der umwelt. (4. mitteilung). einflüsse des bodens, der luft und des lichtes auf die empfänglichkeit der pflanzen für krankheiten. Phytopath. Ztschr. 3: 1–88, illus.
(17) Walker, J. C.
1936. resistance to club root in brassica. (Abstract) Phytopathology 26: 112.
(18) ———
1939. resistance to clubroot in varieties of turnip and rutabaga. Jour. Agr. Res. 59: 815–828.

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